EVALUATING FACTORS THAT INFLUENCE APPETITE AND GLYCEMIC RESPONSE TO WILD RICE AND WILD RICE BLENDS IN HUMANS

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ABSTRACT

Background: Wild rice has been reported to have higher protein, dietary fibre, and phytochemicals compared to white rice and brown rice. It has also shown numerous health benefits in animal models and *in vitro* studies. But no study has reported the effects of wild rice consumption on postprandial (occurring after a meal) appetite and blood glucose response in humans.

Objectives: To investigate the effects of cooked Canadian wild rice and wild rice blends consumption on palatability, postprandial appetite and blood glucose response in adults and evaluate the nutritional components that could be responsible for the study outcomes.

Design: The acute trial followed a randomized crossover controlled design. Participants (n=19, 10 males and 9 females) consumed 140 g of treatment; stovetop cooked wild rice, brown rice, white rice (control), a wild rice blend of 15% wild rice and 85% brown rice, and microwaved wild rice blend with 250 mL water. Their blood glucose concentration and appetite were measured at intervals from 0 to 120 min. Palatability of the treatments was measured following consumption. To explore the potential impact of parboiling on blood glucose results, wild rice was parboiled and nutritional composition of the treatments used in the study and the parboiled wild rice were analyzed and compared.

Results: From the trial, it was observed that the stovetop cooked wild rice had about 32.7% increase in the postprandial blood glucose response when compared to stovetop cooked parboiled white rice ($p \leq 0.05$). No differences were observed for appetite among the treatments. Parboiled white rice (70.7%) and brown rice (72.4%) were more palatable than wild rice (61.3%) and wild rice blends (57.1% for microwave and 64.0% for stovetop). Based on these results we explored

the impact of parboiling on wild rice. We found that parboiling improved the protein, total dietary fibre, amylose, total phenolic content, fat content, and reduced the rapidly digestible starch, slowly digestible starch, starch damage, total flavonoid content, and carbohydrates in wild rice. Also, the cooked parboiled wild rice showed intermediate estimated glycemic index (eGI) of 64 in *in vitro* digestion calculation compared to cooked non-parboiled wild rice with eGI of 77.

Conclusion: This study showed that short-term consumption of 140 g of stovetop cooked non-parboiled wild rice led to higher glycemic response compared to parboiled brown and white rice in adults and no differences were observed for appetite. This is despite wild rice having more protein, fibre and phytochemicals than white rice and brown rice. Parboiling wild rice could be an excellent method to reduce blood glucose response to wild rice consumption, without meaningfully decreasing its positive nutritional properties. Further studies are needed to investigate insulin response following wild rice consumption and clinical trials are needed to investigate the effects of cooked parboiled wild rice on postprandial appetite and blood glucose response.

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DEDICATION

I dedicate this thesis to my amazing husband, Amaechi Chukwu, who has been my backbone since day one. Your unwavering support and love mean everything to me. To our families, who have been my rock throughout this journey, thank you for being there for me. I also want to acknowledge God's guidance and blessings, and my own determination and perseverance, which have carried me through the ups and downs. May the blessings be...

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LIST OF ABBREVIATIONS

Full name	Abbreviations
Visual Analogue Scale	VAS
Postprandial glycemic response	PPGR
Glycemic index	GI
Blood glucose concentration	BGC
Glycemic load	GL
Informed Consent Form	ICF
Pancreatic α-amylase	PAA
Amyloglucosidase	AMG
Research Electronic Data Capture	REDCap
Glucose oxidase/peroxidase reagent	GOPOD
Hour	H
Minutes	Min
Seconds	Sec
Milligram	mg
Microgram	µg
Gram	G
Millilitre	mL
Degree Celsius	^{o}C
Percentage	%
Revolution per minute	rpm

CHAPTER 1: INTRODUCTION

Rice (*Oryza sativa*) is a staple food for over 50% of the world population. White rice and brown rice are differentiated based on the milling process. Brown rice is a whole grain, it consists of its bran layers, embryo, and endosperm which contributes to its high dietary fibre, minerals, vitamins and phytochemicals, thus increasing its health benefits to humans than white rice which loses its bran layer during milling (Carcea, 2021). Yet white rice is preferred and greatly consumed by most of the population (Carcea, 2021). Regular white rice consumption has been reported to be associated with increased risks of developing diabetes (Bhavadharini et al., 2020; Carcea, 2021; Hu et al., 2012).

Diabetes is a serious, chronic metabolic disease reducing the life span of millions of people (Heald et al., 2020). In 2019, 9% of the world's population (463 million adults) had diabetes, in 2021, 10.5% of the world's population (536.6 million people) was estimated to have diabetes and it is expected to increase to 12.2% (783.2 million people) in 2045 (Sun et al., 2022). In Canada, 10% of Canadians were diagnosed with diabetes in 2024 and this is expected to increase to 12% by 2034 (Diabetes Canada, 2024). Type 1 diabetes is a chronic autoimmune disease whereby an individual's immune system destroys the β-cells of the pancreas responsible for producing insulin (Kahaly & Hansen, 2016) and type 2 diabetes is a condition whereby the pancreas does not produce enough insulin and/or the cells are resistant to the insulin produced which results in build-up of glucose in the blood (Chatterjee et al., 2017). Globally, type 2 diabetes has been a great burden in public health service (Zimmet, 2017). Some diabetes complications include cardiovascular disease, diabetic kidney disease, retinopathy and neuropathy which can affect their life quality, result in blindness, kidney failure. and/or death (Cole & Florez, 2020). The role lifestyle and food choices play in the development and management of type 2 diabetes have been documented

previously (Alkhatib et al., 2017; Hu, 2011; Rahati et al., 2014). Therefore, replacing or substituting white rice with healthier rice varieties or species may help reduce diabetes prevalence or complications in people with diabetes.

Wild rice is a member of the genus *Zizania.* There are four known *Zizania* species, three of which are *Zizania aquatica* L., Zizania *texana* Hitche, and *Zizania palustris* L. are indigenous cereals in North America, whereas *Zizania latifolia* (Griseb) Turcz is found in China, Vietnam, and Japan. Wild rice is also commonly known as American rice, Indian rice, Canadian rice, water oat, Menomin, and water rice (Yan et al., 2019; Zhai et al., 2001). *Z. aquatica* and *Z. palustris* are annual crops, while *Z. texana* (perennial crop) is not usually used as food, and it is found in the wild (Porter, 2019). Wild rice in Canada was originally found in the provinces of Manitoba, Quebec, and Ontario (Fyles, 1920). It is found naturally in quiet, shallow, and muddy freshwater to brackish waters or lakes that flow gently in these provinces (Porter, 2019). It can also be cultivated on set-up beds, which is an artificial set-up that has the environmental conditions necessary for the growth of wild rice (Fyles, 1920; Lu et al., 2005; Surendiran et al., 2014). Wild rice is usually harvested, dried naturally under the sun, parched (roast dried), and dehulled (removal of the husk) (Fyles, 1920; Surendiran et al., 2014).

Wild rice has been reported to have higher amounts of protein, dietary fibre, vitamins, phytochemicals, and minerals but low in carbohydrate content when compared to white rice and brown rice (Saleh et al., 2019; Yu et al., 2020; Zhai et al., 2001). Wild rice consumption has been shown to have health benefits in mitigating insulin resistance and lipo-toxicity in rats (Han et al., 2013), prevention of atherosclerosis in mice (Moghadasian et al., 2019; Surendiran et al., 2013) and have anti-hypertensive effect in rat (Deng et al., 2014). *In vitro* studies have shown immunomodulatory, anti-allergic, anti-inflammatory, anti-hypertensive, and antioxidant

properties of wild rice (Qiu et al., 2009, 2010). These effects are attributed to its nutritional composition such as its phytochemicals, high fibre (Zhai et al., 2001) and phenols (Chu et al., 2018; Qiu et al., 2009, 2010).

Most people in North America have little to no knowledge about wild rice and its health benefits despite being grown in this region. Overall, health research with wild rice is limited and very few studies have been done on the postprandial (occurring after a meal) glycemic response to wild rice in animal models and humans (Zhang et al., 2015) and none on appetite (desire to eat food or drink which is usually triggered by hunger) (Marcus, 2013). Hence, this thesis research aims to investigate the effects of wild rice on postprandial appetite and blood glucose response in humans and determine the nutritional factors responsible for the outcome.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Below is a review of the nutritional composition of wild rice, research on postprandial glycemic and appetite response, and factors that may influence those responses to rice.

2.2 Nutritional Composition of Wild Rice

2.2.1 Carbohydrates

The carbohydrate content of wild rice is about 71 - 84% and serves as its main source of energy. Its starch content is about 60 - 65%. The carbohydrate in wild rice has more branched structures, and a dietary fibre of 5.2% (Surendiran et al., 2014) which accounts for the low glycemic index (GI) of 53.72 in Chinese wild rice (Zhang et al., 2015). Glycemic index (GI) is a system (in a scale of 100) that rates foods with available carbohydrates (carbohydrates that are broken down in the small intestine in humans) (McCleary et al., 2020) based on the effect it has on the blood glucose concentration after its consumption (Augustin et al., 2015). Carbohydrate foods are classified into high, medium, and low GI foods. The carbohydrate contents in high GI (range from 70 and above) foods are rapidly digested by the body and results in quicker increase in the blood glucose concentration. On the other hand, low and medium GI foods carbohydrate content are broken down and absorbed into the blood at a much slower rate than that of high GI food. The low GI foods range is ≤ 55 while the medium GI foods is between 56 to 69 (Augustin et al., 2015). However, it is not advisable to use GI alone to determine if a food is healthy or not (Augustin et al., 2015).

Dietary fibres are carbohydrates that cannot be broken down in the human small intestines, so are available for fermentation in the large intestine. Wild rice consists of soluble (0.8%) and insoluble

(3.3%) dietary fibre. These wild rice components have been shown to be associated with the reduction of high postprandial glycemia in animals (Surendiran et al., 2014). Dietary fibre consumption has been associated with lower type 2 diabetes risk (Panahi et al., 2007). Research has shown that replacing carbohydrate sources with others that are higher in fibre and protein can reduce the risk of hyperlipidemia (high lipid content in the blood), hyperglycemia (high blood glucose concentration), and high body mass index (Jiang et al., 2007). Additionally, raw wild rice has a lower carbohydrate content of 75 g/100 g compared to raw white and brown rice which have 79.95 g/100 g and 77.24 g/100 g respectively (U.S. Department of Agriculture, 2013).

2.2.2 Proteins

Zhai et al. (2001) carried out a study to compare the nutritional composition of Chinese and North American wild rice. It was observed that the Chinese and North American wild rice has approximately the same amount of protein which range from 12.0 - 15.15%, and it is two times the protein content found in white rice (7.13%) (Zhai et al., 2001). The amount of protein in North American wild rice is 14.7 g/100 g while that of white rice and brown rice is 7.13 g and 7.94 g/100 g respectively (U.S. Department of Agriculture, 2013). Wild rice is gluten-free and has a higher amount of essential amino acids such as methionine than white rice (Yu et al., 2020; Zhai et al., 2001).

2.2.3 Lipids

Raw wild rice fat content ranges from 0.7 – 1.1% while that of brown rice is 2.7 - 2.9%. Brown rice has twice the lipid content found in wild rice while raw white rice and wild rice have similar fat content, around 0.7% (U.S. Department of Agriculture, 2013). Chinese wild rice has more fat content of about 1.07 g/100 g when compared to American wild rice (0.83 g/100 g) (Zhai et al.,

2001)**.** The major fatty acids found in wild rice include; 35 – 37% linoleic acids, 20 – 31% linolenic acids, 14.1 – 18.4% palmitic acids, 1.1 – 1.3% stearic acids, and 12.8 – 16.2% oleic acids (Przybylski et al., 2009; Yu et al., 2020)**.**

2.2.4 Vitamins

Wild rice is a great source in water-soluble (Vitamin B group such as thiamine and riboflavin) and fat-soluble vitamins (vitamin E; tocopherol and tocotrienols, which contributes to the antioxidant property of wild rice). The thiamine and riboflavin composition are 0.36 – 0.50 mg/100 g and 0.20 mg/100 g in North American wild rice and 0.52 – 0.63 mg/100 g and 0.07 – 0.15 mg/100 g in Chinese wild rice, respectively. Hence, they are greater than those found in white rice (0.12 mg/100 g and 0.05 mg/100 g) (Zhai et al., 2001). Tocopherol in North American wild rice (0.2 mg/100 g) and Chinese wild rice (0.48 mg/100 g) is more than that in white rice (0.1 mg/100g) (Peanparkdee & Iwamoto, 2019; Zhai et al., 2001). Additionally, wild rice is not milled before consumption, hence its nutrients are retained (Yu et al., 2020).

2.2.5 Minerals

The mineral content of wild rice is notably higher than white rice and brown rice as shown in table 1 below.

Table 1: Mineral Constituents of Wild Rice, White Rice and Brown Rice.

Minerals	**Wild rice (mg/100 g)**	**White rice (mg/100 g)**	**Brown rice (mg/100 g)**
Calcium	21.96 - 24.22	4.25 - 12.67	7**.**76 – 49.0
Iron	1.53 - 3.17	0.97 - 3.06	1.96 - 8.88
Magnesium	106.41 - 120.91	1.15 - 21.58	2.46 - 43.74
Phosphorus	236.61 - 384.73	45.67 - 140.16	201.0 - 252.97
Potassium	145.59 - 244.91	35.0 - 96.64	58.5 - 184.36
Zinc	1.25 - 2.83	1.45 - 2.47	1.61 - 5.39
Reference	(Zhai et al., 2001)	(Antoine et al., 2012; Mir et al., 2020)	(Mir et al., 2020)

2.2.6 Phytochemicals

It has also been reported that wild rice has numerous phytochemicals, which are hypothesized to contribute to the health benefits observed with wild rice consumption (Qiu et al., 2009, 2010). Sterols like phytosterols and γ-Oryzanol, γ-aminobutyric acid, and phenol compounds such as phenolic acids and flavonoids are found in wild rice (Qiu et al., 2010). Phytosterols are steroid compounds naturally produced by plants (Shahzad et al., 2017). In wild rice, the total phytosterol content in lipids is about 70 -145 g/kg which is more than the 27 g/kg found in brown rice lipids (Jiang et al., 2016; Przybylski et al., 2009). The types of phytosterols commonly found in wild rice include 19 – 33% β-sitosterol, 14 – 52% campesterol, 5 – 12% cycloartenol, and 5 – 12% Δ^5-avenasterol (Przybylski et al., 2009). Wild rice lipids have more γ-Oryzanol (459–730 mg/kg) than rice bran oil (359 mg/kg) (Przybylski et al., 2009). γ-Oryzanol (mixture of phytosterol ferulates and triterpene alcohol) from rice bran oil (Rice (*Oryza sativa* L.)) has been shown to have neuroprotective, anti-hyperlipidemic, anti-carcinogenic, and anti-inflammatory effects in mice (Lemus et al., 2014; Rungratanawanich et al., 2019). Since wild rice γ-Oryzanol is higher than that in rice bran oil, it was hypothesized that wild rice may possess some of these health benefits (Yu et al., 2020). The well-known types of flavonoids found in wild rice are proanthocyanidins and flavonoid glycosides (Yu et al., 2020) and the most abundant phenolic acids are ferulic and synapic acid (Qiu et al., 2010).

Table 2: Nutritional components of North American Wild Rice, White Rice and Brown Rice

Nutrients	**Wild Rice Raw**	**White rice Long grain Enriched raw**	**Brown rice Long grain Raw**
Energy (kcal/100 g)	357.0	365.0	370.0
Protein (g/100 g)	14.7	7.1	7.9
Lipid (g/100 g)	1.1	0.7	2.9
Carbohydrates (g/100 g)	75.0	79.9	77.2

Dietary fibre (g/100 g)	6.2	1.3	3.5
Moisture (g/100 g)	7.8	11.6	10.4

Table source: (U.S. Department of Agriculture, 2013).

2.2.7 Amylose-amylopectin ratio

Amylose is a polysaccharide made of glucose units. It is a short linear polymer linked by α bonds. Amylopectin is also made of glucose units (arranged in linear with branched α-bonds appearing within 24-30 glucose units) that are branched and have a longer polymer (Sajilata et al., 2006). Higher amylose content makes starch more resistant, so the higher the amylose the lesser the digestibility of the starch and impact on blood glucose concentration (Boers et al., 2015). Amylose content ranges from 0 – 2% is waxy, 10 – 20% is low, 20 – 25% is intermediate and > 25% is regarded as high amylose content starch (Chatterjee & Das, 2018). Zhang et al. (2022) reported amylose content for two Chinese wild rice starch (*Z. latifolia*) as 19.82% and 20.33% (Zhang et al., 2022). 29.4% amylose content was reported in wild rice starch (*Z. Palustris L*) (Hoover et al., 1996), and 18.0 to 21.8% was observed in wild rice starch (*Z. aquatica)* from Minnesota (Wang et al., 2002).

2.3 Postprandial Glycemic Response

Postprandial glycemic response (PPGR) is the measure of the effect a meal or food has on the blood glucose concentration after ingestion (Augustin et al., 2015). To measure postprandial glycemic response, an individual fasts for 10 to 12 hours overnight after which the fasting blood glucose concentration is measured. The individual consumes the investigational product (food, drink, food component), then the blood glucose concentration is determined over two hours and the incremental area under the curve (iAUC) is calculated using trapezoidal rule (Mendes-Soares et al., 2019).

To diagnose whether an individual has impaired glucose response, an oral glucose tolerance test (OGTT) can be conducted. This measures the blood glucose response after 2 hours following the consumption of a standard glucose drink. If after 2 hours blood glucose concentration (BGC) is ≤ 7.8 mmol/L, it is categorized as normal. However, if BGC is between 7.8 and 11.0 mmol/L it indicates prediabetes, and BGC ≥ 11.1 mmol/L indicates diabetes (Care, 2022).

2.4 Blood Glucose Control

Blood glucose control involves maintaining a normal blood glucose concentration. The body secretes hormones (insulin and glucagon) which help to regulate the blood glucose concentration. Insulin is a hormone that helps regulate the blood glucose concentration by allowing the cells to take up glucose from the bloodstream (Dimitriadis et al., 2021). In the first phase of insulin secretion (2 to 10 minutes) following the ingestion of food, insulin is secreted from the beta-cells in the pancreas which limit blood glucose increase and hepatic glucose production in people without diabetes. After which insulin is secreted in the second phase until a normal blood glucose level is reached (Caumo & Luzi, 2004). On the other hand, glucagon is released from the alpha-cells in the pancreas. It helps elevate the blood glucose concentration when it drops below the normal range by stimulating the liver to convert glycogen to glucose which is released into the blood (Briant et al., 2016).

2.5 Glycemic Response to Wild Rice

There are few studies pertaining to the effect of wild rice on blood glucose concentration. Below is a summary of the research conducted on the glycemic response to wild rice.

Zhang et al. (2015) investigated the glycemic index of Chinese wild rice. In this study, eight (8) healthy adults' blood glucose concentrations were measured after the consumption of 67 g of

Chinese wild rice. Their blood glucose concentration was measured within 2 hours after the wild rice consumption. Jenkins and Wolever's formula were used to calculate the glycemic index. 50 g of glucose was used as a control. It was concluded that Chinese wild rice has a low glycemic index of 53.72 (Zhang et al., 2015).

Han et al. (2013) observed the implication of replacing dietary carbohydrate with wild rice (*Zizania latifolia* (Griseb) Turcz) in high-fat diet-fed rats. The goal of the study was to replace the main source of dietary carbohydrates in the city diet (a meal commonly consumed in Asia) which consists of processed wheat starch and white rice with wild rice and then examine its effects on insulin resistance in rats. The city diet was formulated in accordance with the diet consumed by Asian residents which has high saturated fat, cholesterol, and carbohydrates. Ten-week-old male Sprague-Dawley rats were used for this experiment and grouped at random into four treatments; high-fat and cholesterol diet (HFC), City diet (CD), Wild rice (WR), and Low-fat diet (LF). Each treatment group had 10 rats. Only the rats in the Low-Fat diet group were not fed with high fat and cholesterol diets for eight weeks. At the end of the experiment (eight weeks), rats fed with wild rice diet had a lower weight gain and showed a significant reduction in serum fasting insulin and fasting blood glucose concentrations than those fed with HFC and CD. Hence, it was concluded that whole-grain cereal with high bioactive content like wild rice can prevent insulin resistance and decrease chronic metabolic syndrome in rats fed with a diet rich in fat and cholesterol (Han et al., 2013).

A study by Moghadasian et al. (2019) investigated the gut microbiome, cytokines, and metabolomics after wild rice consumption in low-density lipoprotein receptor knockout (LDL-r-KO) mice. The authors observed a 60% increase in the plasma glucose concentration of mice fed with wild rice compared to the control mice fed with white rice. No explanation was provided for

this outcome despite the fact that wild rice has high dietary fibre and protein (Moghadasian et al., 2019). This contradicts the previous study by Han et al. (2013) and our general knowledge on the role of dietary fibre and protein in food on glycemic response.

Table 3: Summary of Research on Glycemic Response to Wild Rice

Study	Objectives	Conclusions	References
In vivo study of Chinese wild rice in humans	Determine glycemic index	Chinese wild rice has a low glycemic index of 53.72	Zhang et al., 2015
In vivo study of wild rice on high fat and cholesterol diet-fed rats	Improve insulin resistance	Chinese wild rice prevents insulin resistance and decrease chronic metabolic syndrome in wild rice fed rats	Han et al., 2013
In vivo study of wild rice in LDL-r-KO mice	Anti-atherosclerotic ability	Anti-atherogenic property was seen in the wild rice fed mice (higher interleukin-10 and erythropoietin in the plasma) but 60% increase in the plasma glucose level was observed	Moghadasian et al., 2019

2.6 Factors that Influence Postprandial Glycemic Response to Rice

Evaluating the factors that influence glycemic response to wild rice by comparing it to white rice could provide an in-depth knowledge of the various nutritional components that influence glycemic response. Unfortunately, little research has been done with wild rice, so understanding the factors that determine glycemic response to other grain such as white rice (which is known) can help us identify factors which should be evaluated in relation to glycemic response to wild rice consumption. Boers et al. (2015) outlined the effects of rice characteristics and its processing methods on postprandial glycemic response in a systematic review. They identified several factors

that influence or determine the degree of glycemic response to rice, including the starch characteristics of the food (amylose: amylopectin ratio), the post-harvest processing (parboiling) and the final processing before consumption such as cooking type, cooking time, reheating, storage (Boers et al., 2015). Some of them are explained below;

2.6.1 Starch Characteristics

Amylose is a polysaccharide made of glucose units. It is a short linear polymer linked by α-1,4 glycosidic bonds. Amylopectin is a highly branched long polymer linked by α-1,6 glycosidic bonds consisting of glucose units (Sajilata et al., 2006). Starch resists digestion when the amount of amylose is high. Therefore, higher amylose content makes starch more resistant, so the higher the amylose the lesser the digestibility of the starch (Boers et al., 2015). Therefore, the amount of resistant starch (starch that is not digested by enzymes in the human small intestine within 4 hours, but it is broken down in the large intestine (McCleary et al., 2020)) in a food influences the absorption of starch and consequently reduces the glycemic response.

2.6.2 Protein and Lipid Content

Fat reduces the rate in which glucose is absorbed, hence, decreasing blood glucose peak to the ingested food (Sheard et al., 2004)**.** It was suggested that protein increases insulin production when consumed with carbohydrates. This may result in reduction in glucose in the blood (Sheard et al., 2004)**.** Although the effect of protein and fat in food may be different from diet to diet. Protein (amino acids, enzymes, and nucleic acids) found in food may inhibit starch hydrolysis which reduces the digestibility of the food and blood glucose response. Monounsaturated and polyunsaturated fatty acids are present in starchy foods, and they are said to improve palatability. Lipids in food form complexes with starch, this reduces the digestion rate and lowers the

postprandial blood glucose response (Lal et al., 2021). Ye et al. (2018) reported that lipid and protein cause low GI in *Indica* rice cultivars by forming a coat on the surface of the starch granule which prevents the starch from swelling (Ye et al., 2018), therefore slowing digestion (Lal et al., 2021; Ye et al., 2018)**.**

2.6.3 Phytochemicals

Phytochemicals (polyphenols such as phenolic acids and flavonoids) are chemical substances produced by plants which protect them against microorganisms' infections. Research, both *in vitro* and *in vivo* have demonstrated the chemo-preventive, anti-inflammatory, antioxidative, and neuroprotective potentials of plant-based polyphenol-rich foods. These plant-based compounds have been suggested to influence the metabolism of carbohydrates by reducing carbohydrates digestibility, glucose absorption and increasing insulin secretion in the body. Hence, this may result in a decrease in the postprandial and fasting blood glucose response. Also, flavonoid and phenolic acids have been suggested to inhibit the activities of digestive enzymes such as amylase (Hanhineva et al., 2010).

2.6.4 Gelatinization

Gelatinization is the transformation of starch granules into gel by heating the starch in water. This also results in a change in the starch structures and properties. The higher the gelatinization temperature, the more rapidly digestible starch (RDS), and the less resistant starch (RS) it contains (Chung et al., 2006). This change in the structure affects the digestibility of cooked rice. Rice varieties cooked for a shorter time retained the amount of amylose in them. Therefore, there may be no difference in the digestion of the rice starch as the process of gelatinization was incomplete (due to shorter cooking time) (Boers et al., 2015). Also, the mechanism of cooking in microwave

is rapid than stovetop hence there may be little changes in food nutritional and physical component (Khalid et al., 2023). This could have an impact on the glycemic response.

2.6.5 Retrogradation

The recrystallization of amylose and amylopectin is called retrogradation. This results in changes in the physical characteristics of the food such as higher viscosity, turbidity of pastes, and gel formation (Hoover et al., 2010). Low starch digestibility rate was seen in cold storage of starch (keeping rice in refrigerator after cooking), and re-ordering of molecules in the starch (Li et al., 2014). The cooling allows the dissociated starch molecules to reassemble. The higher the amount of long linear amylose chains, the lower the rate of digestion and its effect on the blood glucose concentration (Cai et al., 2021).

2.6.6 Post-Harvest Processing

The post-harvest processing steps in paddy rice such as the milling (removal of the husk and bran layer from the paddy), parboiling and quick cooking all affect the nutrients and digestibility of rice. In parboiling, the grain is soaked, steamed, dried, and dehulled. Here, the crystalline structure in the rice is converted to unstructured form and the starch undergoes gelatinization and retrogradation in this process (Boers et al., 2015)**.** Numerous studies have reported that parboiled rice helps to regulate the blood glucose concentration than non-parboiled rice both in healthy participants and people with diabetes (Hamad et al., 2018; Pathiraje et al., 2011).

2.6.7 Viscosity of Food

Viscosity measures a fluid resistance to flow under a set condition (Jin et al., 2023). Viscosity plays an important role in digestion and absorption of nutrients in foods. The higher the viscosity of the food, the lower the rate of digestion and absorption of the nutrients in the small intestine

(Karthikeyan et al., 2019). It has also been reported that high viscous foods help to regulate gastric emptying, satiety, appetite, and postprandial blood glucose response than the consumption of low viscous foods (Argyrakopoulou et al., 2020; Jin et al., 2023).

2.7 Appetite

Appetite is a physiological drive to eat food which is usually triggered by hunger (Marcus, 2013). Satiety refers to the sensation of fullness after a meal which could prolong or inhibit the intake of energy at a later meal. It is a state of being satisfied; not willing to eat (Mollard et al., 2012; Stubbs et al., 2000). Appetite and satiety are two interconnected physiological responses, with appetite driving the desire to eat and satiety signaling the feeling of fullness after a meal.

Satiety can be determined using food intake or subjective ratings of appetite including hunger and fullness. Usually, the visual analogue scale (VAS) is used to measure appetite. The VAS has horizontal line or scale of 100 mm, with description at both ends of the line. For instance, the first point may be labelled "very hungry" and the other "very full." This allows the participants to adjust the scale to either direction based on their appetite and the distance from the start point to the participant's indicated mark is calculated. The VAS can be used to ask the participants a variety of questions about their appetite and palatability of the treatment (Gibbons et al., 2019; Mollard et al., 2012; Stubbs et al., 2000). Also, gastric peptides associated with satiety and appetite such as cholecystokinin (CCK), peptide YY (PYY), glucagon-like peptide 1 (GLP-1), which stimulates satiety and ghrelin (hunger hormone - released in the stomach. it stimulates appetite) can be used as biomarkers to measure appetite. Practically, there are many difficulties associated with measuring satiety peptides. This procedure is expensive, strenuous and the peptides degrade extremely fast (Gibbons et al., 2019).

2.8 Factors that Influence Postprandial Appetite Response to Rice

Similar factors influence postprandial appetite and satiety to food. These have been categorized into sensory, cognitive, post-ingestive and post-absorptive factors by Blundell et al. (1987) in satiety cascade (Blundell et al., 1987).

Sensory and cognitive factors include the perception about the food, palatability or taste, food texture and smell which can influence appetite (Benelam, 2009; Blundell et al., 1987). It was suggested that solid and semi-solid food increases satiety than their liquid counterpart. The same report was given for high viscous foods (slows down hunger due to reduced gastric emptying rate) than low viscous foods. Therefore, for high viscous foods to cause a slower rate of gastric emptying (rate at which food leaves the stomach), the energy load of the food should be high (Stribiţcaia et al., 2020).

After the ingestion of food, the food gets to the stomach and causes it to expand (gastric stretching). This stretching of the stomach is a post-ingestive factor and it sends signals to the brain indicating its fullness. The greater the amount of food consumed, the more the stomach stretches. As digestion continues to take place, hormones that aid satiety are released from the intestine (Benelam, 2009; Blundell et al., 1987). The more viscous the intestinal content is, the more time it spends in the intestine and decreases the rate nutrients are absorbed. This enhances interactions between the intestinal wall and the nutrients which stimulate the release of peptides (Ghrelin, CCK, GLP-1, PYY) that regulate appetite (Rebello et al., 2016).

Blood glucose, insulin, and amino acids concentrations control appetite in the post-absorptive phase. These nutrients are detected by the brain, and then it gives more information about the nutritional state of the body (Amin & Mercer, 2016; Benelam, 2009; Blundell et al., 1987).

Also, the nutritional composition of the food is another factor that can influence appetite and satiety (Amin & Mercer, 2016). Macronutrients such as carbohydrates, protein and fat have been suggested to be associated with satiety. Previous studies have reported that protein gives a higher satiating effect followed by carbohydrates and fats (Anderson et al., 2004; Benelam, 2009; Cho et al., 2013; Rebello et al., 2016). Rice based foods with high protein and fibre content have been suggested to improve fullness hence reducing hunger (Slavin & Green, 2007). Fibre increases volume and has low energy density hence plays an important role in long-term fullness. It requires more time to chew, which slows the rate of absorption of nutrients thus resulting in satiety and reducing appetite. Food with high dietary fibre may slow down gastric emptying and stimulate gastric stretching. Therefore, whole grain consumption and food rich in fibre may help reduce appetite (Cho et al., 2013; Rebello et al., 2016). Since wild rice is higher in protein and dietary fibre and lower in carbohydrates, it may be more satiating than white rice. However, not all fibre has been shown to be associated with increased satiety as some fibre is supplemented into the diet while others occur naturally in the food (Slavin & Green, 2007). Protein and fat also reduce the rate at which food leaves the stomach (gastric emptying). This is because fat breaks down more slowly than other nutrients. Veldhorst et al. (2012) concluded that the consumption of diet with higher protein content increases satiety in healthy people (Veldhorst et al., 2012). Decreased hunger and postprandial blood glucose response has been shown to be associated with diets with high protein and fibre content (Chan et al., 2019; Mollard et al., 2012, 2014). Micronutrients such as calcium and some vitamins may indirectly help control satiety (Major et al., 2008).

Individual behavior or characteristics such as chewing habit, eating rate, perception about the food, the quantity of food and spacing between meals may influence appetite (Blundell et al., 2010; Rebello et al., 2016; Woods, 2004). Although the postprandial appetite response to wild rice has

not been evaluated in previous studies, it is therefore important to determine appetite response to wild rice.

CHAPTER 3: RATIONALE, OBJECTIVES, AND HYPOTHESES

This research trial investigates the effects of cooked Canadian wild rice and wild rice blends on postprandial appetite and blood glucose response in humans is part of a larger project titled "An Indigenous Strategy for Re-Energizing Traditional Wild Rice: Indigenous Wealth Creation" funded through Protein Industries Canada (PIC). This PIC project is led by the Myera Group, which is a Manitoba based Métis company that is developing novel, sustainable biotechnology for producing high-value products that will have a positive impact on human health.

3.1 Rationale

Wild rice has been reported to have higher protein, dietary fibre, vitamins, phytochemicals, minerals, and lower carbohydrate content, when compared to white rice and brown rice (Saleh et al., 2019; Yu et al., 2020; Zhai et al., 2001). These nutrients have been shown to help regulate glycemic response and appetite (Clarke et al., 2022; Weickert & Pfeiffer, 2018). Also, wild rice consumption has shown numerous health benefits in animal models; such as mitigating insulin resistance and lipo-toxicity in rats (Han et al., 2013), preventing atherosclerosis in mice (Moghadasian et al., 2019; Surendiran et al., 2013), and has anti-hypertensive effect in rats (Deng et al., 2014). In *in vitro*, it has shown to have immunomodulatory (Wang et al., 2018), anti-inflammatory, anti-allergic (Lee et al., 2015), and antioxidant properties (Qiu et al., 2009, 2010; Sumczynski et al., 2017). To our knowledge, no study has reported appetite response to wild rice and there are limited studies on blood glucose response and glycemic index of wild rice in humans (Zhang et al., 2015). Due to gaps in the literature, there is a need to investigate the impact of wild rice consumption on postprandial glycemic and appetite response in humans and evaluate the nutritional factors that may influence the outcomes. This is also supported by the fact that wild rice is native to North America (it grows naturally in Canada) and has been consumed by the

Indigenous communities for thousands of years. Additionally, the increased prevalence of diabetes in Canada and in the world, and concern to regulate blood glucose concentration with food choices is another drive for this research (Diabetes Canada, 2024; Sun et al., 2022).

The result of this research will help inform the development of blended rice products that include wild rice and may help inform food choices in terms of selecting types of rice for people who want to control their blood glucose response.

3.2 Research Objectives

The objectives of this research are to:

1. Investigate the effects of cooked Canadian wild rice and wild rice blend on postprandial appetite and blood glucose response compared to white rice and brown rice in humans.
2. Evaluate the nutritional factors that may be responsible for differences seen in postprandial appetite and blood glucose response.
3. Investigate if cooking method (stovetop and microwave) impacts postprandial appetite and glycemic response.

3.3 Research Hypotheses

The hypotheses are as follows;

1. Cooked whole wild rice would have a lower postprandial blood glucose response and appetite rating in humans compared to cooked white rice, brown rice, and a blend of 15% wild rice and 85% brown rice (wild rice blend).

2. The nutritional composition of wild rice such as the protein, dietary fibre, phytochemicals content etc. would influence the appetite and glycemic response in humans and would be responsible for the outcomes seen.

3. The wild rice blend prepared using microwave would have a lower postprandial glycemic response in humans compared to wild rice blend cooked on stove top.

CHAPTER 4: CLINICAL TRIAL - ACUTE EFFECTS OF WILD RICE (*ZIZANIA PALUSTRIS*) CONSUMPTION ON APPETITE AND BLOOD GLUCOSE RESPONSE: RESULTS OF A CROSSOVER RANDOMIZED CONTROLLED TRIAL

4.1 Abstract

Wild rice (WR) is a grain native to North America. It has been reported to have higher protein, dietary fibre, phytochemicals, and lower carbohydrate content compared to white rice (WhR) and brown rice (BR). Several animals and *in vitro* studies have reported health benefits associated with WR consumption, but no study has reported postprandial appetite and blood glucose responses to wild rice in humans. This trial investigated the effects of cooked wild rice on postprandial glycemic and appetite responses in humans. Following a randomized crossover design, 19 participants completed this trial in Winnipeg, Manitoba. They consumed 140 g treatments (stovetop cooked WR, BR, WhR, a wild rice blend (WRB) of 15% WR and 85% BR, and microwaved WRB) with 250 mL water. Blood glucose concentrations were measured using finger stick blood sampling, and visual analogue scales (VAS) were used to measure appetite at intervals from 0 – 120 min and palatability following treatment consumption. Unexpectedly, stovetop cooked parboiled white rice consumption led to a 32.7% lower blood glucose response when compared to the stovetop cooked wild rice, which was not parboiled ($p \leq 0.05$). No differences were observed for appetite. Parboiled WhR and BR were rated as more palatable than WR and WRB. These findings highlight the potential of cooked wild rice as a nutritious alternative to white rice and further investigation into insulin response, and parboiling process on postprandial glycemic response to wild rice. This outcome will help people make informed choices on the type of rice to eat.

Keywords: Wild rice, wild rice blends, blood glucose response, glycemic response, satiety, appetite, palatability, postprandial blood glucose response, human trial

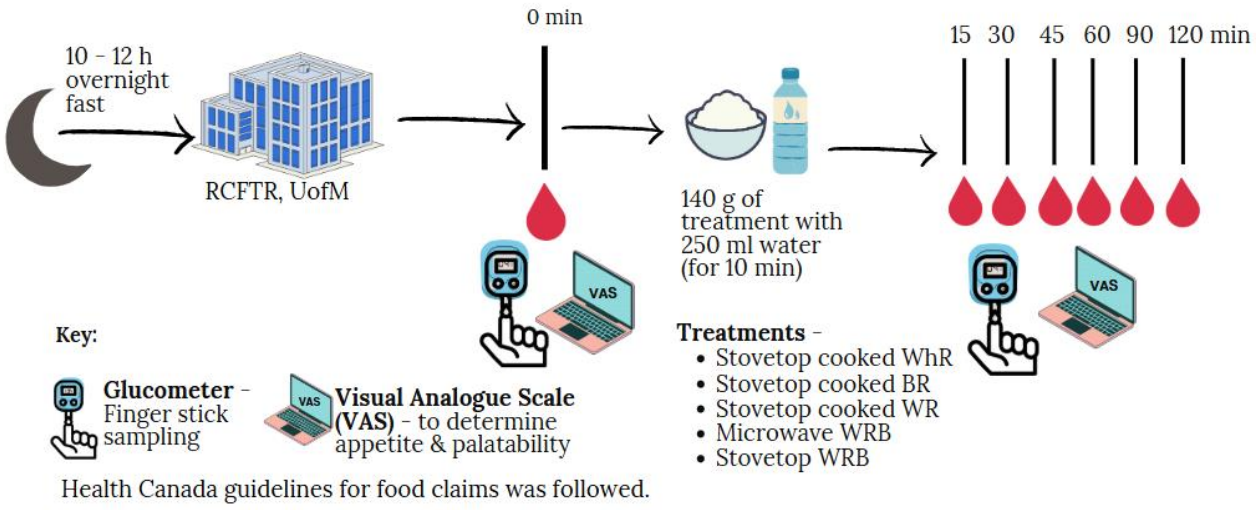

Figure 1: Graphical Representation of Study Design (Created in Canva)

4.2 Introduction

Rice is a staple food for over 50% of the world population. In 2014, about 408 million metric tons of milled rice was consumed globally per year (Muthayya et al., 2014). Brown rice is a whole grain, it consists of its bran layers, embryo, and endosperm which contributes to its high dietary fiber, minerals, vitamins and phytochemicals, thus increasing its health benefits to humans than white rice. Yet white rice is preferred and greatly consumed by most of the population (Carcea, 2021). Regular white rice consumption has been reported to be associated with increased risks of developing diabetes (Bhavadharini et al., 2020; Carcea, 2021; Hu et al., 2012). In 2021, 10.5% of the world's population (536.6 million people) was estimated to have diabetes and expected to increase to 12.2% (783.2 million people) in 2045 (Sun et al., 2022). Similarly in Canada in 2024, it is estimated that 10% of Canadians have been diagnosed with diabetes, and it is expected to increase to 12% by 2034 (Diabetes Canada, 2024).

There are four known wild rice (*Zizania* species) *Zizania* species, three of which are *Zizania aquatica* L., Zizania *texana* Hitche, and *Zizania palustris* L. are indigenous grain in North America, whereas *Zizania latifolia* (Griseb) Turcz is found in Asia (Yu et al., 2020). Wild rice is also sometimes called American rice, Indian rice, Canadian rice, water oat, Menomin, and water rice (Yan et al., 2019; Zhai et al., 2001). The US Food and Drug Administration recognized wild rice as a whole grain in 2006 (Surendiran et al., 2014). Wild rice in Canada was originally found in the provinces of Manitoba, Quebec, and Ontario and has been consumed by Indigenous communities for thousands of years (Fyles, 1920). It has received interest due to its nutritional composition and health benefits (Yu et al., 2020). Wild rice has been reported to have higher protein, dietary fibre, vitamins, phytochemicals, minerals, and lower carbohydrate content, when compared to white rice and brown rice (Saleh et al., 2019; Yu et al., 2020; Zhai et al., 2001). Its consumption has shown numerous health benefits in animals, such as it mitigates insulin resistance and lipo-toxicity in rats (Han et al., 2013), prevents atherosclerosis in mice (Moghadasian et al., 2019; Surendiran et al., 2013), has anti-hypertensive effects in rat (Deng et al., 2014). *In vitro* studies have suggested immunomodulatory (Wang et al., 2018), anti-inflammatory, anti-allergic (Lee et al., 2015), and antioxidant properties (Qiu et al., 2009, 2010; Sumczynski et al., 2017). To our knowledge, no study has reported appetite; the desire to eat food or drink which is usually triggered by hunger (Marcus, 2013), and limited studies have reported blood glucose response and glycemic index of wild rice in humans (Zhang et al., 2015).

Objective 1: To investigate the effects of cooked Canadian wild rice (*Zizania palustris*), and a blend of 15% wild rice and 85% brown rice (wild rice blend) consumption on postprandial blood glucose, appetite, palatability responses compared to parboiled white rice and parboiled brown rice in humans.

Objective 2: To investigate if cooking method (stovetop compared to microwave) impacts postprandial glycemic and appetite responses to the wild rice blend in humans.

From the existing literature, we hypothesized that cooked whole wild rice would have a lower postprandial glycemic response and appetite rating (feeling of hunger) in humans compared to cooked white rice, brown rice, and wild rice blends, since it has higher protein, dietary fibre and phytochemicals than the other rice. The microwave wild rice blend would have a lower blood glucose and appetite rating responses compared to the stovetop cooked wild rice blend as the mechanism of cooking in microwave is more rapid (involves heating food from inside to outside with less cooking time) than stovetop. This may result in lesser changes in the nutrient and the food physical structure (Khalid et al., 2023).

4.3 Materials and Methods

4.3.1 Treatments

The rice products; Canadian wild rice (*Z. palustris*), long grain parboiled brown rice, long grain parboiled white rice, and wild rice blend (15% Canadian wild rice and 85% long grain parboiled brown rice) were provided by the Floating Leaf Fine Foods Inc. Sunnyside, MB, Canada**.** The rice products were stored and prepared in the Richardson Centre for Food Technology and Research (RCFTR) metabolic kitchen at the University of Manitoba where the trial took place.

Note: The 15% wild rice blend is the percentage of wild rice which will be made available by the company for their commercial sales.

There were five study treatments;

- Stovetop cooked white rice (Stp WhR)

- Stovetop cooked brown rice (Stp BR)
- Stovetop cooked wild rice (Stp WR)
- Microwaved wild rice blend (Mic WRB) and
- Stovetop cooked wild rice blend (Stp WRB)

4.3.2 Cooking Procedure

The cooking procedure of the rice products was in line with the manufacturer's instruction with little adjustments to ensure that no water was drained out of the cooked rice. The methods of cooking (stovetop and microwave) were chosen as they are readily available to people.

4.3.2.1 White Rice

In a pot, one cup of water was added to 75 g of raw white rice. It was brought to boil on medium high heat stovetop for 5 - 6 minutes. Then the heat was reduced to medium low and covered to cook for 20 minutes.

4.3.2.2 Brown Rice

In a pot, one and quarter cups of water was added to 65 g of raw brown rice. It was brought to boil on medium high heat stovetop for 5 - 6 minutes. Then the heat was reduced to medium low and covered to cook for 30 minutes. After which the heat was turned off and allowed to stand on the hot surface for 10 - 12 minutes.

4.3.2.3 Wild Rice

In a pot, one and quarter cups of water was added to 65 g of raw wild rice. It was brought to boil on medium high heat stovetop for 5 - 6 minutes. Then the heat was reduced to medium low and

covered to cook for 30 minutes. After which the heat was turned off and allowed to stand on the hot surface for 30 minutes.

4.3.2.4 Wild Rice Blend (Microwave)

In a microwave-friendly bowl, two cups of water were added to 100 g of raw wild rice blend. It was microwaved for 20 minutes with constant stir within each 5 minutes interval.

4.3.2.5 Wild Rice Blend (Stovetop)

In a pot, one and quarter cups of water was added to 65 g of raw wild rice blend. It was brought to boil on medium high heat stovetop for 5 - 6 minutes. Then the heat was reduced to medium low and covered to cook for 30 minutes. After which the heat was turned off and allowed to stand on the hot surface for 25 - 30 minutes.

The cooking procedure was maintained throughout the study. 140 g of the study treatments with 250 ml of water were given to the participants in a randomized order in each session. Randomization was done automatically on the Research Electronic Data Capture (REDCap) platform.

4.3.3 Analysis of Rice Samples and Study Treatments

The proximate analysis performed on the raw rice samples (wild rice, white rice, brown rice, and wild rice blend) were done by SGS Canada Inc. BC, Canada. Moisture (AOAC 935.29), protein (AOAC 990.03), iron, sodium and potassium (AOAC 2011.14), total sugar (AOAC 982.14), carbohydrates (water method), total fat (AOAC 996.06) (Thiex, 2009). Total Phenolic content, total flavonoid content, starch damage, amylose-amylopectin ratio, resistant starch, rapidly digestible and slowly digestible starch, and total dietary fibre of raw and cooked samples, and fat,

protein, moisture content, and viscosity of study treatments were analyzed at various laboratories in the University of Manitoba.

4.3.3.1 Samples Preparation

The study treatments were cooked as described previously in section 4.3.2, freeze-dried, and milled through 0.5 mm sieve while the raw rice samples were milled through 0.5 mm sieve.

4.3.3.2 Moisture Content Analysis

The moisture content was determined as described in AACC method 44-15. The oven was preheated to 130 ^{o}C. Cooked rice sample (1-2 g) was weighed into the dish. Samples in the dish were placed in a 130 ^{o}C preheated oven for 1 h. After 1 h, the dishes were transferred into a desiccator to cool for 30 min. After 30 min, the dish containing the samples were removed from the desiccator and weighed on the same scale. The weight of dish or container, samples before and after drying was recorded in grams (AACC, 2000). Analysis was done in duplicate.

% moisture = (container + sample before drying – container + sample after drying)/ (container + sample before drying – container weight) * 100

4.3.3.3 Measurement of Protein Content

The protein content of the study treatments was measured using Dumas (Combustion) method; AOAC 990.03 method (Thiex, 2009). Approximately 200 mg of milled freeze-dried samples were weighed into foil. The foil was sealed properly and placed in an N analyzer (Elementar Rapid N EXCEED, Model: APSA-370), and allowed to run. After which the percentage of protein in each sample was displayed (Bicsak et al., 1993; Thiex, 2009). Analysis was done in duplicate.

4.3.3.4 Measurement of Total Ash Content

Samples (3 g) were weighed into dish in duplicate. The samples were placed in a cold muffle furnace oven at 575 ^{o}C for 12 h overnight. After which the samples were allowed to cool down and transferred in a desiccator for 1 h. The ash was weighed and recorded (AACC, 2000).

4.3.3.5 Measurement of Total Dietary Fibre

Dietary fibre was measured using ANKOM Fibre analyzer as described in AACC 32-07/AOAC 991.43 (AACC, 2000). Samples (0.5 g) were weighed and transferred into insoluble dietary fibre (IDF) bags in ANKOM Fibre analyzer and started following the manufacturer's instruction. It automatically sends in the appropriate reagents at the time it is needed. After which the bags were dried in oven for 90 min, ash and protein contents were determined. Total dietary fibre content was calculated by adding percentage of soluble and insoluble dietary fibre (AACC, 2000).

4.3.3.6 Measurement of Total Phenolic and Total Flavonoid Content

4.3.3.6.1 Preparation of Extract

The phenolic contents in the samples were extracted in duplicate as described by Apea-Bah et al. (2022) (Apea-Bah et al., 2022). Each milled sample (100 mg) was weighed into different 2 mL amber-colored microcentrifuge tube (to prevent exposure of the mixture to light) and 1 ml of 80% methanol (100 mg/mL) was added to each tube and vortexed. The mixture was sonicated using Branson Ultrasonic Cleaner (Model: 5510R - DTH) for 60 min to extract the phenolic compounds. After which the tubes were centrifuged at 20,000 x g for 5 min to collect the supernatant which contains the phenolic compounds into clean tubes. The extract was used for total phenolic and flavonoid content analysis.

4.3.3.6.2 Total Phenolic Content Analysis

The Total Phenolic Content of the study treatments and raw rice samples were estimated using the Folin-Ciocalteu Method (Singleton & Rossi, 1965) as described by (Apea-Bah et al., 2022). Stock standard solution of gallic acid (Sigma–Aldrich Chemical Co. (St. Louis, MO, USA)) (1 mg/mL) were prepared by weighing 1 mg of gallic acid into a 2 mL microcentrifuge tube. Then 1 mL of 50% methanol was added to the gallic acid, and the tube was vortexed and sonicated for 5 min to ensure the complete dissolution of the solid. After that, different concentrations (0, 12.5, 25, 50, 75, 100 and 150 µL/mL) of gallic acid calibration solutions from the gallic acid stock solution were prepared using 50% methanol to get 500 µL for each concentration. The control tube contained only 500 µl of 50% methanol. Sample extracts (18.2 µL) and gallic acid standard (18.2 µL) was transferred into different well in a 96-well microplate. Then 36.4 µL of 10% aqueous Folin-Ciocalteu reagent (v/v) was added to the extracts and standard in the microplate well. Sodium Carbonate (Na_2CO_3) (145.5 µL of 700 mM of Na_2CO_3) was added to each well and the mixture was mixed and incubated at room temperature for 1 h. The absorbance was read at 750 nm using BioTek (ELx800) Spectrophotometer (Apea-Bah et al., 2022). The standard curve was plotted (Gallic acid standard curve equation $x = (y - 0.0092)/0.0095$, and $R^2 = 0.9997$), and the total phenolic content was expressed in Gallic acid Equivalent (GAE) mg/100 g sample.

4.3.3.6.3 Total Flavonoid Content Analysis

The analysis of the Total Flavonoid Content in the study treatments and raw rice samples were performed using the Aluminum Chloride Colorimetric Method as described by (De Souza et al., 2014). Standard stock solution was prepared by dissolving 1.3 mg of Rutin (Quercetin-3-β-D-rutinoside from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA)) (1 mg/mL) with 26 µL of Dimethyl Sulfoxide (DMSO) and then 1274 µL of double distilled water (ddH_2O) was added. The

tube was ultrasonicated for 5 min to ensure it dissolves completely. The calibration standard solutions were prepared through serial dilution of Rutin standard stock solution with 1 mL of ddH_2O in six (6) tubes to obtain the following concentrations 400, 200, 100, 50, 25, and 12.5 µg/mL. Different concentrations of Rutin calibration standards (400 µl) or sample extracts (400 µl) or ddH_2O (for blank) (400 µl) was transferred into 5 mL tubes, 80 µL of 5% Sodium nitrite ($NaNO_2$) was added into the tubes and it was shaken and allowed 6 min to stand. Aluminum chloride ($AlCl_3$) (80 µL of 10% $AlCl_3$) was added and allowed 6 min to stand. After 6 min, 800 µL of 10% Sodium hydroxide (NaOH) (g/v) was added and 640 µL of ddH_2O was added to make the volume 2 mL. The tubes were vortexed and allowed to stand for 15 min. After that, the mixture was transferred to cuvette and the absorbance was measured at 510 nm using Thermo-scientific (GENESYS 30) Visible **S**pectrophotometer against the blank solution (De Souza et al., 2014). The standard curve was plotted (Rutin standard curve equation $x = (y + 0.0044)/0.0014$ and $R^2 = 0.9997$), and outcome was expressed in Rutin Equivalent (RE) mg/100 g sample.

4.3.3.7 Measurement of Crude fat Content

The crude fat of the study treatments was measured using Soxhlet extraction method in duplicates. The drying oven was preheated to 125 ^oC and 250 mL flat bottom flasks containing 5 boiling chips were placed in the preheated oven for 30 min. After that, the flasks were removed from the oven and allowed to cool in desiccator. Then the flasks were weighed (w_o). Milled samples (w_s) (3 - 4 g) were weighed into filter paper which was properly folded and placed into porous paper thimble and wad of glass wool was used to cover the sample in the thimble. Each thimble was placed into a Soxhlet extraction tube. In a fume hood, 150 mL of hexane was poured into each 250 mL flat bottom flask. Then the flat bottom flasks were directly placed below the corresponding extraction tube containing the sample. The condenser turned on and apparatus was turned on and it was

allowed to extract at the rate of 2 - 3 drops per second condensation for 16 h (overnight). After that, the flat bottom flasks were placed on 3 Recess Model heating mantle and heated to evaporate the remaining hexane, leaving behind the extracted fat. Then the flasks were dried in preheated drying oven for 1 h at 100 ^{o}C. After 1 h, the flasks were transferred to cool in desiccator. Then the weight of the flask with extracted fat (w_f) was measured using analytical balance (Thiex, 2009). The crude fat was calculated using the formula: % crude fat = $\{(w_f - w_o) * 100\}/w_s$

Where; w_o = weight of preheated flat bottom flask with boiling chips (g), W_s = weight of sample (g), and W_f = weight of flask and fat after drying (g).

4.3.3.8 Carbohydrate Calculation

The total carbohydrate in the samples were calculated using the formula below:

% of Carbohydrates = 100% - (% of Ash) - (% of Fat) - (% of Moisture) - (% of Protein) (Kanzler et al., 2015).

4.3.3.9 Starch *In vitro* Digestion Procedure

The *in vitro* digestion was done following the method described by Santamaria et al. (2021) and Benavent-Gil & Rosell (2017) (Benavent-Gil & Rosell, 2017; Santamaria et al., 2021). Samples (200 mg) were dissolved in 4 mL of 0.1 M sodium maleate buffer (pH 6.9) with porcine pancreatic α-amylase (0.9 U/mL) (Sigma Chemical, St. Louis, USA) and were incubated in a shaking water bath at 37 °C for 180 min. Aliquots of 100 μL were taken at 0, 5, 10, 20, 30, 40, 60,75, 90, 105, 120, 150, 180 min, and mixed with 100 μL of 96% ethanol to terminate the enzymatic hydrolysis. The blank contained 200 mg of sample with 4 mL of 0.1 M sodium maleate buffer (pH 6.9) and was incubated in a shaking water bath at 37 °C for 180 min. Aliquots of 100 μL were taken at 0 and 180 min and were mixed with 100 μL of 96% ethanol. After which, samples were centrifuged

at 10,000 × g and 4 °C for 5 min. Then the pellet was washed with 100 μL of 50% ethanol and the supernatants were stored for further glucose determination. Supernatant (100 μL) was diluted with 855 μL of 0.1 M sodium acetate buffer (pH 4.5) and incubated with 15 μL amyloglucosidase (AMG) (241.5 U/mL) at 50 °C for 20 min in a shaking water bath. It was centrifuged at 2000 × g for 10 min, and supernatants were used for glucose determination.

After 16 h of hydrolysis, the remaining starch were homogenized with 2 mL of 2 M KOH using a Polytron Ultraturrax homogenizer IKA-T18 at 14,000 rpm for 1 min. Then 8 mL of 1.2 M sodium acetate (pH = 3.8) and 100 μL AMG (143 U/mL) were added and tubes were incubated at 50 °C in a shaking water bath for 30 min. It was centrifuged at 2000 × g for 10 min to obtain the supernatant for glucose determination. Megazyme glucose oxidase–peroxidase (GOPOD) kit was used to measure the glucose content and absorbance was read using a spectrophotometer at 510 nm. Starch was calculated as glucose (mg) × 0.9. The starch fraction hydrolyzed within 20 min of incubation were the rapidly digestible starch (RDS), between 20 and 120 min is the slowly digestible starch (SDS), and after 16 h total digestible starch (TDS). After 16 h of incubation, the unhydrolyzed starch is the resistant starch (RS). The hydrolysis index (HI) was calculated by dividing the area under hydrolysis curve of the sample (0–180 min) by the area of the sample more concentrated (1:4) over the same period. The expected glycemic index (eGI) was calculated using the equation; eGI = 8.198 + 0.862HI (Benavent-Gil & Rosell, 2017; Santamaria et al., 2021).

4.3.3.10 Amylose/Amylopectin Analysis

Amylose/Amylopectin ratio was determined using the Amylose/Amylopectin Megazyme kit (Megazyme International Ireland Ltd). Milled samples (35 mg) and 25 mg of the Megazyme high amylose maize starch (68%) were weighed into 15 mL glass test tubes. Dimethyl sulfoxide (DMSO) (1 mL) was added to the tubes and vortexed simultaneously. The tubes were placed in

water bath at 100 ^{o}C for 15 min, after every 5 min, the tubes were vortexed vigorously. After 15 min, the tubes were removed from the water bath and allowed to stand for 5 min at room temperature. Then 2 mL of 95% ethanol was added, and tubes were vortexed and additional 4 mL 95% ethanol was added, vortexed, and allowed to stand for 5 min at room temperature for complete precipitate formation to form. After that, the tubes were vortexed and centrifuged at 1500 x g for 10 min. The supernatant was discarded, and tubes were inverted on paper towel for 10 min to ensure complete removal of ethanol. DMSO (2 mL) was added to the pellet in the tubes and vortexed simultaneously. The tubes were placed in water bath at 100 ^{o}C for 15 min, after every 5 min, the tubes were vortexed vigorously. After 15 min, the tubes were removed from the water bath and 4 mL of Concanavalin A (Con A) solvent (from the Megazyme kits) was added and vortexed immediately. Then the content in the tubes were transferred to a 25 mL volumetric flask and the volume was made 25 mL. The solution (2 mL) was transferred into 2 mL microcentrifuge tubes and were centrifuged at 10,000 x g for 3 min (Schirmer et al., 2013).

For Amylose determination, 1 mL of the solution was transferred into a 2 mL microcentrifuge tube and 0.5 mL Con A solution was added, mixed gently and allowed to stand for 1 hour at room temperature. The tubes were centrifuged at 14,000 x g for 10 min. After which 1 mL of the supernatant was transferred into 15 mL glass tubes. Sodium acetate buffer (3 mL of 100 mM) with pH 4.5 was added to the glass tubes and tubes were heated in water bath at 100 ^{o}C for 5 min to denature the Con A. To equilibrate, the tubes were placed in water bath at 40 ^{o}C for 5 min. Amyloglucosidase/alpha-amylase enzyme mixture (100 µL) and incubated at 40 ^{o}C for 30 min. It was vortexed and aliquot was transferred into 2 x 2 mL microcentrifuge tubes and tubes were centrifuged at 2000 x g for 5 min. Then 1 mL of the supernatant was transferred in duplicate into 10 mL disposable glass test tubes and 4 mL of GOPOD was added and tubes were incubated at 40

^{o}C for 20 min. Absorbance was read at 510 nm against the reagent blank which consisted of 1 mL 100 mM sodium acetate buffer + 4 mL GOPOD and D-glucose control consisted of 0.1 mL 1 mg/mL glucose + 0.9 mL sodium acetate buffer + 4 mL GOPOD (Schirmer et al., 2013).

For total starch determination, 0.5 mL of solution A was added to 10 mL disposable glass tubes and about 4 – 5 mL of 100 mM sodium acetate buffer (pH 4.5), 100 µL of amyloglucosidase/alpha-amylase solution was added, vortexed and incubated at 40 ^{o}C for 10 min. 1 mL was transferred in duplicate into 10 mL disposable glass test tubes and 4 mL of GOPOD was added and tubes were incubated at 40 ^{o}C for 20 min. Absorbance was read at 510 nm against the reagent blank (Schirmer et al., 2013). % Amylose was calculated using the formula below:

Amylose % = (Absorbance amylose determination x 66.8)/ Absorbance Total Starch Aliquot.

Amylopectin % = 100 – Amylose %

4.3.3.11 Measurement of Starch Damage

Starch damage refers to physical breakdown of the starch granules of a food. Starch damage was measured using the Megazyme Assay Kit for starch damage (K-SDAM 06/18) which follows the AACC Method 76-31.01 and ICC Method No. 164 (Gibson et al., 1993). Milled study treatments (20 mg) were weighed into 15 mL glass KIMAX tube. The tubes and α-amylase solution in a separate plastic centrifuge tube were equilibrated for 5 min and 10 min respectively at 40 ^{o}C in water bath. After which, 1.0 mL of the pre-equilibrated α-amylase solution was added to each tube, vortex for 5 sec, and incubated for 10 min at 40 ^{o}C. After incubation, 8.0 mL of dilute sulphuric acid solution (0.2% v/v) was added to each tube and stirred thoroughly to inactivate the enzyme and bring the reaction to an end. The tubes were centrifuged at 1000 x g for 10 min. Then 100 µL of the supernatant was transferred to the bottom of disposable culture tube in duplicate. After

which, 100 µL of amyloglucosidase solution was added to each disposable culture tube and vortexed for about 5 sec. The tubes were incubated for 10 min at 40 ^{o}C in water bath. 4.0 mL of GOPOD reagent solution was introduced to each test tube and incubated again at 40 ^{o}C for 20 min. The absorbance of the solutions was measured at 510 nm against a reagent blank. The starch damage of the treatment will be calculated using;

SD% = E x F/W x 8.1

Where E = absorbance read against reagent blank, F = 150 mg/ (Absorbance of 150 µg of glucose), W = weight, mg, and 8.1 = conversion factor (Megazyme booklet).

4.3.3.12 Measurement of Viscosity

Viscosity measures a fluid's quality or resistance to flow under a set condition. The study treatments were prepared as described in section 4.3.2. They were grated for 30 sec in a blender to reduce the particle size. Then homogenized in batches in plastic tube (Since 140 g of treatment and 250 mL of water was consumed in the trial, for 13 g of grated sample, 23 mL of water was added) using homogenizer (ULTRA-TURRAX IKA T18 Basic) until about 300 mL of the solution was collected in 400 mL beaker in triplicate at room temperature. The viscosity of the study treatment solution was performed using Brookfield Dial Viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with LV spindle 63, and suitable speed in revolution per minute (rpm) for each sample (Faustino et al., 2015). The dial readings were recorded for each sample after 5 rotations. The viscosity was calculated using the formula:

Viscosity in centipoise (cP) (mPa's) = Dial reading x Factor (from the factor table for LV series viscometer provided by Brookfield. The factor considers the speed and spindle used).

4.3.3.13 Calculation of Energy

The nutritional composition of the treatments measured in dry weight were calculated to get it estimated wet weight in 140 g. The total energy of 140 g of each study treatment was calculated using the Atwater System. The percentage of protein, carbohydrate and fat in the treatments were multiplied with their respective energy factor and summed to get the total energy (Capuano et al., 2018).

Table 4: Nutritional Composition of Raw White Rice, Brown Rice, Wild Rice, and Wild Rice Blends

Nutrients	Raw wild rice	Raw wild rice blend	Raw brown rice	Raw white rice
Crude protein content (%)	12.8	8.7	8.0	7.8
Carbohydrates content (%)	76.1	74.8	76.8	80.5
Total dietary fibre (%)	4.4	4.0	3.0	0.9
Total fat (%)	0.9	2.6	2.7	0.9
Moisture content (%)	8.6	12.6	11.3	10.0
Total sugar content (g/100 g)	1.2	1.0	0.5	0.4
Energy (Cal/100 g)	359	350	355	349
Crude ash content (%)	1.6	1.2	1.2	0.8
Cholesterol (mg/100 g)	<2.0	<2.0	<2.0	<2.0
Sodium (mg/100 g)	2.0	<1	2.0	1.0
Iron (mg/100 g)	1.7	1.3	1.2	1.2
Potassium (mg/100 g)	346.0	247.0	222.0	163.0
Amylose (%)	31.9	30.1	36.4	34.7
Amylopectin (%)	68.1	69.9	63.6	65.3
TPC (mg GAE /100 g)	46.9	32.8	33.3	18.1
TFC (mg RE/100 g)	267.8	151.7	55.3	29.6

Key: TPC – Total phenolic content, GAE – Gallic Acid equivalent, TFC – Total flavonoid content, RE – Rutin equivalent. Results presented in the table is in dry basis (db)

Table 5: Percentage of the nutritional components in 140 g of each study treatment

Nutrients (%)	Cooked white rice	Cooked brown rice	Cooked wild rice	Microwave wild rice blend	Stovetop wild rice blend
Protein	3.6	3.2	5.4	5.3	3.9
Carbohydrate	33.3	27.2	33.2	39.2	26.0
Fat	0.7	1.2	1.4	3.1	2.0
Moisture	62.1	68.0	59.4	51.7	67.6
Ash	0.3	0.4	0.6	0.7	0.5
TDF	0.7	1.2	2.1	2.1	1.7
SDS	13.7	10.4	13.4	23.0	11.9
RDS	7.4	5.1	9.2	13.8	7.5
RS	3.5	2.5	3.1	7.6	3.7
TPC (GAE)	0.1	0.1	0.2	0.2	0.1
TFC (RE)	0.2	0.3	1.2	0.6	0.5
Energy (Cal/140 g)	153.9	132.4	167.0	205.9	137.6
Starch damage (% db)	57.8	60.8	52.7	44.2	57.2
Amylose (% db)	32.1	31.0	35.9	34.9	33.9
Amylopectin (% db)	67.9	69.0	64.1	65.1	66.1
eGI	69.0	63.0	77.0	90.0	69.0
Viscosity (Pa*s)	68.8	5.9	1.2	18.3	2.1

Key: SDS – Slowly digestible starch, RDS – Rapidly digestible starch, RS – Resistant starch, TDF – Total dietary fibre, eGI – Estimated glycemic index**,** TPC – Total phenolic content, GAE – Gallic Acid equivalent, TFC – Total flavonoid content, RE – Rutin equivalent, Pa*s – Pascal-seconds, Cal - Calorie, db – dry basis.

4.3.4 Clinical Trial Design

4.3.4.1 Ethics

The Biomedical Research Ethics Board (BREB) at Bannatyne Campus of the University of Manitoba, Winnipeg, MB, Canada approval was received; HS25900 (B2023:033) before commencing this research and Good Clinical Practices (GCP) was followed. This research has been registered in clinicaltrials.gov (NCT05976633). Participants consented to this study by reading and signing the informed consent form before the screening session.

4.3.4.2 Sample Size

Sample size of 20 used in this study was estimated to give 97% power ($1-\beta = 97\%$) in 5 sessions (P. G. Sun, 2010) to detect a difference of 22 mmol/L x min in the incremental area under the curve (iAUC) blood glucose reduction (one sided) using an estimated intra-participant standard deviation of 16% (from previous clinical trials from this lab group on the acute effects of some products on glycemic response; both published (Johnston et al., 2021a, 2021b) and those yet to be published) for the 5 sessions. The 22 mmol/L reflects a 20% reduction in iAUC, which was used based on the difference proposed by Health Canada to support reduction in postprandial glycemic response claims (Braunstein et al., 2018).

4.3.4.3 Participant Inclusion and Exclusion Criteria

Individuals with a normal fasting blood glucose concentration between 3.5 - 5.6 mmol/L, within 18-50 years, and had body mass index (BMI) range of 18.9 – 29.9 kg/m^2 were recruited in Winnipeg, Manitoba, Canada. Individuals who do not consume breakfast, are allergic to rice, have diabetes, have a history of hypertension, pregnant and/or nursing, have participated in another research intervention in the past 12 weeks prior to this study commencement, on medications that may influence glucose metabolism, or have ongoing gastrointestinal diseases were excluded from this study. Participants' baseline data such as age, fasting blood glucose, and BMI were reported in mean ± standard deviation.

4.3.4.4 Recruitment, Consent and Screening

Participants were recruited using a combination of local advertisement, including emails circulated to University of Manitoba email address holders, and posters placed in and around the University

of Manitoba, Winnipeg, Canada. Also, advertisements were circulated on social media platforms (LinkedIn and Facebook). The informed consent form was read and signed by interested individuals, and a screening session was organized to determine their eligibility. Prospective participants' weight (kg) and height (m) were measured to calculate their BMI (Kg/m^2), and their fasting blood glucose concentration (mmol/L) were measured using finger stick blood sampling and a StatStrip® glucometer (Nova Biomedical, Mississauga, ON, Canada).

4.3.4.5 Study Design

Health Canada Draft Guidance Document on Food Health Claims related to the Reduction in Postprandial Glycemic Response, and Health Claims on Food on Satiety were followed. There were five (5) sessions in total. In this crossover randomized control trial, participants fasted for about 10 - 12 hours overnight and arrived at the RCFTR between 7:00 am – 11:00 am on the session day. Each session lasted for 2.5 hours. Alcohol was avoided 24 hours before study session. The treatments were randomly assigned to each participant in different sequences by REDCap platform and all study data were recorded in REDCap. At 0 min, the fasting blood glucose concentration of the participants were measured twice via finger stick using StatStrip® glucometer. This was performed throughout the whole study, right before they consumed the study treatments, and at 15, 30, 45, 60, 90 and 120 min after the consumption of the treatments in duplicate. Appetite visual analogue scale (VAS) was used to measure appetite (fullness and hunger), and palatability visual analogue scale (computerized 100 mm scale) was used to determine palatability (how well the participants liked the taste, texture and how pleasant the study treatments were). VAS questionnaire was completed by the participants before the consumption of the treatments to measure appetite (at 0 min) and after the consumption of the treatments to measure palatability (at 15 min), and appetite at several intervals similar to that of the blood

glucose concentration measurement intervals throughout 120 minutes (Flint et al., 2000; Mollard et al., 2012, 2014). At least 3 days were allowed between sessions. Participants who were menstruating were scheduled during the follicular phase of their menstruation cycle because some hormonal imbalances have been observed to occur during the luteal phase (Brennan et al., 2009).

4.3.4.6 Statistical Analysis

Statistical analysis was performed using the Statistical Analysis System (SAS version 9.4). The sessions and sex of the participants were included in the model as fixed factors to detect any session by treatment or sex by treatment interactions. From the appetite VAS questionnaire, the average subjective appetite was calculated as: appetite score = (desire to eat + hunger + (100 – fullness) + prospective consumption)/4 (Anderson et al., 2002; Mollard et al., 2012). This reflected the questions on the questionnaire. Postprandial responses were measured by blood glucose incremental area under the curve (iAUC) and the total area under the curve (tAUC) was calculated for appetite. iAUC and tAUC were calculated using the trapezoidal rule for 0 - 120 minutes, and differences between the study treatments were examined by repeated measures analysis of variance (ANOVA). Repeated measures ANOVA using the PROC MIXED model was employed to analyze the effects of treatments, time, and time-by-treatment interactions on postprandial blood glucose and appetite. Then, to determine differences between the study treatments, repeated measures ANOVA at a particular time was used. Palatability of the treatments were measured using palatability VAS and calculated using the formula; Palatability = (taste + texture + pleasant)/3 (Braunstein et al., 2018; Mollard et al., 2014). Tukey-Kramer post-hoc test was used to compare treatments and Dunnett test was used to compare each study treatment to the control; white rice. Statistically significant at $p \leq 0.05$.

4.4 Results

4.4.1 Participants Characteristics

Participants (n = 20; males n = 10 and females n = 10) were recruited in Winnipeg, MB, Canada. One participant withdrew from the study for personal reasons after completing one session and data was not included. A female participant completed 4 sessions out of 5 before leaving the country and another female participant stopped without formal notice after completing two sessions (data were included). Therefore, total participant n = 19 (males n = 10, females n = 9) were included in the analysis.

Table 6: Participants Baseline Data

	Males (10)	**Females (9)**
Mean age (years)	33.1 ± 5.5	28.3 ± 6.3
Body Mass Index (kg/m^2)	25.0 ± 2.5	24.5 ± 1.5
fasting blood glucose (mmol/L)	4.9 ± 0.5	4.8 ± 0.3

Results are presented in mean ± standard deviation

4.4.2 Blood Glucose Response

Differences were observed between time ($p < 0.0001$), and time by treatment ($p < 0.0001$), but no differences were seen between treatment ($p = 0.141$), sex ($p = 0.726$), and session ($p = 0.373$) with the Tukey-Kramer test. Figure 2 shows the time by treatment interaction on blood glucose response from 0 – 120 min. From 0 – 15 min and some minutes before 30 min of rice consumption, cooked white rice showed higher blood glucose peak and the lowest was observed in cooked wild rice (Figure 2). At 45 and 60 min, wild rice was higher than brown rice $p = 0.019$ and $p = 0.018$ respectively. There was an effect of treatment ($p = 0.032$) on blood glucose iAUC, but no effect in

sex (p = 0.630) or session (p = 0.646). Wild rice had a higher iAUC than the white rice (control), p = 0.049 using a Dunnett-Hsu test (Table 7).

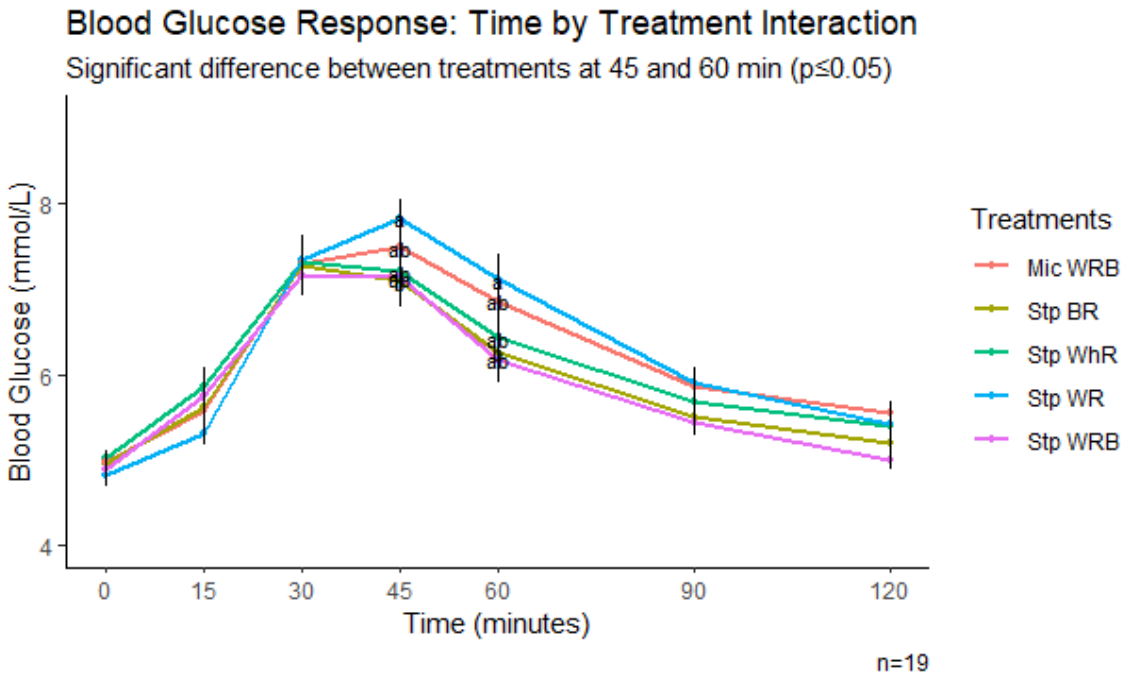

Figure 2: Blood Glucose Response Showing Time by Treatment Interactions (created using R)

Table 7: Blood Glucose Incremental Area Under the Curve, Appetite Total Area Under the Curve and Palatability for each Study Treatment

Treatments	BGR (iAUC)	Appetite (tAUC)	Palatability (mm)
White rice	140.4 ± 15.4[b]	6854.3 ± 443.5	70.7 ± 4.1[a]
Brown rice	132.7 ± 19.1[b]	6782.5 ± 438.4	72.4 ± 3.8[a]
Wild rice	186.3 ± 20.8[a]	6485.6 ± 452.6	61.3 ± 4.2[b]

Microwave wild rice blend	161.9 ± 18.7[b]	5965.7 ± 418.4	57.1 ± 3.6[b]
Stovetop wild rice blend	133.4 ± 17.8[b]	6535.3 ± 380.3	64.0 ± 4.0[ab]

Note: All the values on the table are represented in Mean ± Standard error of mean (SEM) with n = 19. BGR – Blood glucose response, iAUC – incremental area under the curve from 0 – 120 min, tAUC – total area under the curve from 0 – 120 min. For BGR iAUC each treatment was compared to the control; white rice. Values with different lowercase superscript in the same column indicates significant difference at $p \leq 0.05$.

4.4.3 Appetite Response

Differences were observed between time ($p < 0.0001$), and treatment ($p = 0.0009$), but no differences were seen for time by treatment ($p = 0.268$), sex ($p = 0.516$), and session ($p = 0.554$) with the Tukey-Kramer test. Figure 3 shows the time by treatment interaction on appetite response from 0 – 120 min. The differences in treatments were seen between stovetop white rice and microwave WRB ($p = 0.015$), stovetop brown rice and microwave WRB ($p = 0.047$), and microwave WRB and stovetop WRB ($p = 0.045$). There were no differences observed in the tAUC for appetite among the treatments ($p = 0.091$), session ($p = 0.660$), and sex ($p = 0.672$) (Table 7).

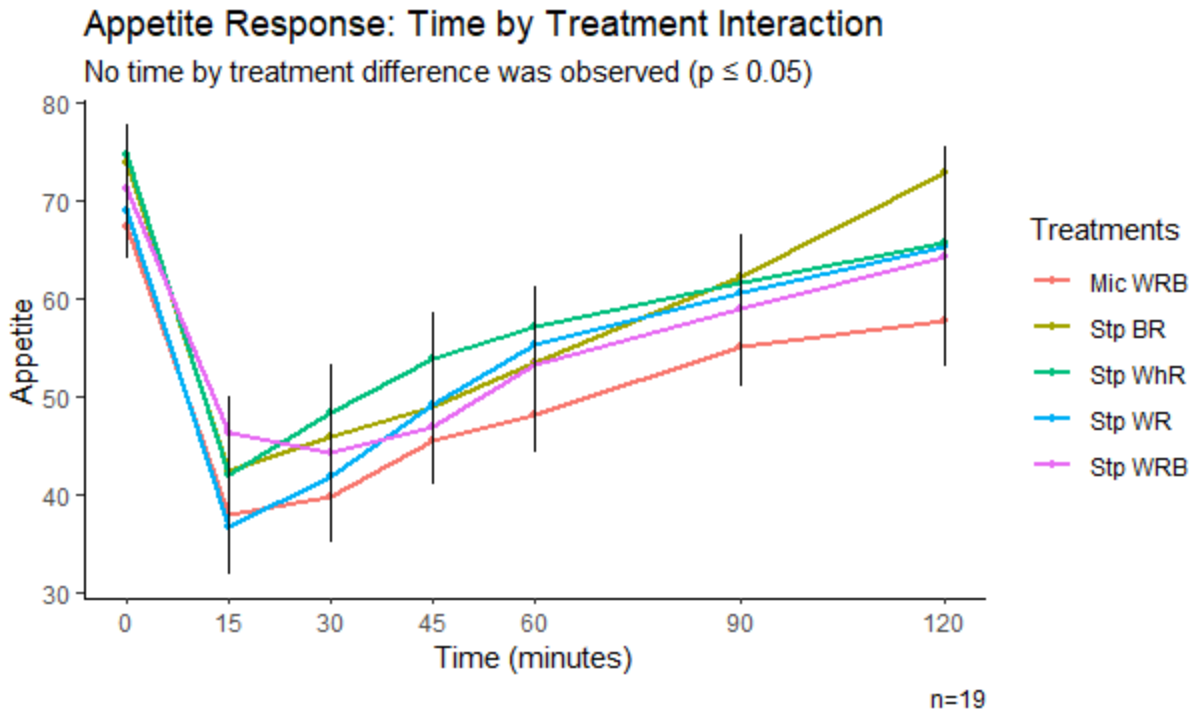

Figure 3: Appetite Response Showing Time by Treatment Interactions (created using R)

4.4.4 Palatability

There was treatment effect (p = 0.002), however, no sex (p = 0.823) and session (p = 0.180). The Tukey-Kramer test showed differences between white rice and wild rice (p = 0.037), white rice and microwave wild rice blend (p = 0.010), brown rice and wild rice (p = 0.034), and brown rice and microwave wild rice blend (p = 0.032). The stovetop wild rice blend was not different from the white rice and brown rice, and wild rice and microwave wild rice blend as shown in Table 7.

4.5 Discussion and Conclusion

In this trial, the postprandial glycemic and appetite responses to cooked Canadian wild rice and wild rice blends in humans were tested. For the blood glucose response, this trial showed that acute

consumption of stovetop cooked wild rice resulted in elevated blood glucose response compared to parboiled white rice, brown rice, and blend of wild rice and brown rice. It was also rated as less palatable than stovetop cooked parboiled white rice and brown rice. There were no differences in appetite ratings among the different treatments.

4.5.1 Effects of the Nutritional Components of Study Treatments on Postprandial Glycemic and Appetite Response

The role high protein and dietary fibre play in regulating blood glucose response and appetite have been reported previously (Clarke et al., 2022; Weickert & Pfeiffer, 2018). The raw wild rice (WR) used in this study has higher protein (12.8%), dietary fibre (4.4%), TPC (46.9 mg GAE/ 100 g), and TFC (267.8 mg RE/ 100 g) than raw white rice (WhR), brown rice (BR) and wild rice blend (WRB) (Table 4). The percentage of nutritional composition presented in raw wild rice is similar to previous research (Massaretto et al., 2023; Moghadasian et al., 2017; Przybylski et al., 2009; Qiu et al., 2009, 2010; Zhai et al., 2001). Some protein can help control the rate of glucose absorption and insulin production in the body (Sheard et al., 2004). When comparing the percentage of the nutritional composition of 140 g of the study treatments, it was observed that cooked WR has higher protein (5.4%) than cooked WhR (3.6%) yet had the highest postprandial blood glucose response.

Phytochemicals have been suggested to influence the metabolism of carbohydrates by reducing carbohydrates digestibility, glucose absorption and increasing insulin secretion in the body. Hence, this may result in a decrease in the postprandial and fasting blood glucose response (Hanhineva et al., 2010). In this study, the percentage of TPC and TFC in 140 g of cooked wild rice (0.2% and 1.2%) were higher than that in cooked parboiled white rice (0.1% and 0.2%) in table 5 respectively

but lower blood glucose response was observed following the consumption of stovetop cooked white rice. Therefore, other components may be responsible for this outcome.

Carbohydrate content in cooked wild rice was 33.2% and it was similar to that in 140 g of cooked white rice (33.3%) (Table 5). The microwave wild rice blend had the highest percentage of carbohydrate content (39.2%) and the 26.0% was observed in the stovetop cooked wild rice blend. Despite cooked WR and WhR having the same amount of carbohydrate consumed, the cooked wild rice showed higher postprandial blood glucose response than cooked white rice and the wild rice blends. Starch resists digestion when the amount of amylose is high. Therefore, higher amylose content makes starch more resistant, so the higher the amylose the lesser the digestibility of the starch (Boers et al., 2015). The microwave WRB had the highest resistant starch content of 7.6%, and the least was seen in cooked brown rice (2.5%). Contrary to Zhang et al. (2022) who observed resistant starch of 15.3% in gelatinized Chinese wild rice starch (Zhang et al., 2022). The microwave WRB had the highest rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) compared to the other study treatments. This may be due to the high amount of carbohydrate in the microwave WRB. The stovetop cooked WR has higher RDS (9.2%) than cooked WhR (Table 5). These may have also contributed to the high blood glucose response seen with wild rice consumption as RDS are starch digested within 20 min and can rapidly increase the postprandial glycemic level (Miao et al., 2015).

The amylose content in cooked wild rice (35.9%), microwave wild rice blend (34.9%), and stovetop wild rice blend (33.9%) is slightly higher than those seen in stovetop cooked white rice (32.1%) and cooked brown rice (31.0%). Higher amylose content makes starch more resistant, so the higher the amylose the lesser the digestibility of the starch and impact on blood glucose concentration (Boers et al., 2015). However, white and brown rice with the least amylose content

have lower blood glucose response than wild rice, hence other factors may have influenced the outcome. Amylose content ranges from 0 – 2% is waxy, 10 – 20% is low, 20 – 25% is intermediate and > 25% is regarded as high amylose content starch (L. Chatterjee & Das, 2018). Although, based on their amylose content, all the samples are high amylose (> 25%). Zhang et al. (2022) reported amylose content for two raw Chinese wild rice starch as 19.82% and 20.33% (Zhang et al., 2022) which is lower than that in Canadian wild rice used in this study. 29.4% amylose content was reported in wild rice starch (*Z. Palustris L*) (Hoover et al., 1996).

The cooking methods; microwave and stovetop used for the wild rice blend did not result in the reduction of the nutrients in the treatments except in the amylose content of stovetop cooked white rice (from 34.7% to 32.1%), and brown rice (from 36.4% to 31.0%) where a slight decrease was observed. Instead, slight increase was in the amylose content of stovetop cooked wild rice (from 31.9% to 35.9%), microwave WRB (from 30.1% to 34.9%) and stovetop WRB (from 30.1% to 33.9%) (Table 4 and 5). Also, the microwave WRB has less starch damage (44.2%) compared to the stovetop WRB (57.2%) and other treatments (Table 5). Hence it is unlikely the nutritional composition of the treatments alone can explain why the postprandial glycemic response to cooked wild rice is higher.

4.5.2 Effects of Cooked Wild Rice and Wild Rice Blends on Postprandial Blood Glucose Response

This present study shows that the short-term consumption of stovetop cooked wild rice increased the blood glucose response in adults (32.7%), producing higher blood glucose peak than stovetop cooked parboiled white rice despite similar amount of carbohydrate was consumed. From figure 2, at 0 – 15 min and some minutes before 30 min of rice consumption, cooked parboiled white rice

showed higher blood glucose peak than cooked wild rice even though it is not different at $p \leq 0.05$. Then at 45 and 60 min, wild rice showed the highest blood glucose peak compared to brown rice. In the first phase of insulin secretion (2 to 10 minutes) following the ingestion of glucose containing food, insulin is secreted from the beta-cells in the pancreas which limit blood glucose increase and hepatic glucose production in people without diabetes. Insulin is a hormone that helps regulate the blood glucose concentration by allowing the cells to take up glucose from the bloodstream. After which insulin is secreted in the second phase until a normal blood glucose level is reached (Caumo & Luzi, 2004), Therefore, the amount of insulin produced (excess or insufficient) in the first phase depends on the amount of glucose in the blood which has influence on the postprandial blood glucose concentration (hypoglycemia or hyperglycemia respectively) (Dimitriadis et al., 2021). This may explain why cooked wild rice had a lower blood glucose response from 0 to 15 min and was the highest at 45 and 60 min. A limitation in this trial is that insulin response was not measured. Therefore, studies are required to investigate the insulin response following cooked wild rice consumption.

Zhang et al. (2015) investigated the glycemic index (GI) of Chinese wild rice (*Z. latifolia*) in a study that involved eight (8) healthy adults who consumed 67 g of Chinese wild rice, and 50 g glucose was used as control. It was observed that Chinese wild rice has a low glycemic index of 53.72 (Zhang et al., 2015). In this present study, the estimated glycemic index (eGI) of the cooked WR following the *in vitro* digestion analysis calculation specified by Santamaria et al. (2021) was 77.0. With this eGI, this cooked WR will be classified as high glycemic index food and that of the stovetop cooked white rice (69.0), brown rice (63.0) and wild rice blend (69.0) would be grouped as medium GI (Table 5). Glycemic index (GI) is a system (in a scale of 100) that rates foods with available carbohydrates based on the effect it has on the blood glucose concentration after its

consumption (Augustin et al., 2015). High GI foods (≥ 70) are rapidly digested by the body and result in quicker increase in the blood glucose concentration. On the other hand, low and medium GI foods carbohydrate content are broken down and absorbed into the blood at a much slower rate than that of high GI food. Therefore, does not cause blood glucose spikes. The low GI foods range is ≤ 55 while the medium GI foods is between 56 to 69 (Augustin et al., 2015)**.** The lowest eGI was seen in cooked BR (63.0) and the highest was in microwave WRB (90.0). The high carbohydrate content in 140 g of microwave WRB may have contributed to its high eGI. From this analysis, since the cooked WR and the microwaved WRB have higher eGI (above 70), it is expected that they show similar high glycemic response. But in this case, the microwave wild rice blend with eGI of 90 showed a lower blood glucose response in the study participants compared to the stovetop cooked wild rice and it's not different from the blood glucose response in cooked white rice. Although, since the eGI was calculated using *in vitro* digestion methods and the previous (Zhang & Zhai, 2016) was *in vivo*, this may have affected the GI outcome. Moreover, the wild rice varieties and the preparation method used in both studies were different.

The stovetop cooked 140 g of wild rice has similar percentage of carbohydrate, higher protein, dietary fibre content and phytochemicals than cooked parboiled white rice, yet it showed high postprandial glycemic response. Therefore, the nutritional components alone of food cannot be used to predict its effect on blood glucose concentration. Other factors like post-harvest processing, insulin response or other factors that can affect ingested foods could be considered. Wild rice is not usually refined or processed unlike many conventional grains (Surendiran et al., 2014). Several studies have reported that parboiled rice can help regulate the blood glucose response than non-parboiled rice in both healthy people and people with diabetes (Hamad et al., 2018; Pathiraje et al., 2011). This may be the case in this study as the white rice and brown rice used were parboiled.

Parboiling is a hydrothermal post-harvest processing of rice, which involves soaking, steaming and drying of paddy before the removal of the husk. This process causes the gelatinized starch granules to reassemble (retrogradation) during drying, therefore reducing the starch digestibility, impact on blood glucose concentration, and improving its palatability and shelf life (Balbinoti et al., 2018). Parboiled white rice is common in supermarkets across Canada and many other countries. According to Peres et al. (2023) parboiling wild rice by gelatinizing the grain in an autoclave at optimal conditions (at pressure of 52.95 kPa for 4.78 min) can improve wild rice quality (bioactive compounds) and reduce its starch digestibility (Peres et al., 2023). This may help reduce the blood glucose response to wild rice seen in this study. Therefore, further studies could be done using parboiled wild rice.

Viscosity measures a fluid resistance to flow under a set condition (Jin et al., 2023). Viscosity plays an important role in digestion and absorption of nutrients in foods. The higher the viscosity of the food, the lower the rate of digestion and absorption of the nutrients in the small intestine (Karthikeyan et al., 2019). It has also been reported that high viscous foods help to regulate gastric emptying, appetite, and postprandial blood glucose response than the consumption of low viscous foods (Argyrakopoulou et al., 2020; Jin et al., 2023). In this study, the viscosity of the study treatments and water consumed were measured using Brookfield Dial Viscometer. It was observed that cooked white rice had the highest viscosity of 68.8 pa*s, and the least viscosity was in cooked wild rice (1.2 pa*s). The high viscosity in WhR may be due to the starchy content in the rice which could easily form gel with water when mixed and homogenized. The microwave WRB viscosity (18.3 pa*s) is higher than the stovetop WRB viscosity (2.1 pa*s) because the rice grains absorbed less water when cooked in microwave than on stovetop. This offered them the ability to thicken when mixed and homogenized in water. Although, this viscosity measurement did not take into

consideration the impact of salivary secretion and gastric secretion on the viscosity of the food during digestion. The degree of chewing, amount of saliva secretion, gastric movement, and the amount of gastric juice secretion can affect viscosity of food and its digestion rate (Jin et al., 2023).

4.5.3 Effects of Cooked Wild Rice on Appetite in Humans

Despite 140 g of cooked wild rice having slightly higher protein (5.4%), and dietary fibre (2.1%) compared to the cooked white rice (3.6% and 0.7%) respectively, no differences were observed among the study treatments for appetite (Table 7). These nutritional components have been reported to increase fullness and reduce food intake for some period than foods with lesser amount of those macronutrients (Akhlaghi, 2024; Kohanmoo et al., 2020). To our knowledge this present research is the first to investigate the effect of wild rice on appetite. Also, the quantity of food consumed by an individual, intervals between meals and individual characteristics are other factors that can affect how full or hungry an individual feels after a meal (Boers et al., 2015). The different cooking methods (stovetop and microwave) of the wild rice blend had no measurable effect on postprandial blood glucose response and appetite in this study.

4.5.4 Palatability of Cooked Wild Rice and Wild rice Blends

The overall acceptability of food can be affected by several factors such as individual characteristics, food sensory properties, if they have consumed the food previously, and preconceived notion about the food type (Murray & Baxter, 2003). Massaretto et al. (2023) reported 87% acceptance of wild rice cooked using an electric rice cooker (*Z. aquatica*) by tasters in their study (Massaretto et al., 2023), while the stovetop cooked wild rice (*Z. palustris*) used in this study had acceptance or palatability of 61.3% (Table 7). For the wild rice blends (WRB), Microwaved WRB had palatability of 57.1% which is lower than that of the stovetop cooked WRB

(64%). The stovetop cooked WRB had the highest palatability rating among all wild rice containing treatment and it was not different from white rice (70.7%) and brown rice (72.4%). Wild rice has crunchy and chewy texture compared to other rice, these characteristics could have affected its palatability rating as rice is known and perceived to be soft and less chewy.

In conclusion, this study showed that short-term consumption of 140 g of stovetop cooked wild rice is associated with about 32.7% increase in the blood glucose response compared to stovetop cooked parboiled white rice in adults despite it having better nutritional profile and same amount of carbohydrate consumed. There were no differences observed for appetite among the treatments. Also, there was no difference in the cooking methods (stovetop and microwave) used for wild rice blends on postprandial glycemic response and appetite, despite the microwave wild rice blend containing 50% more carbohydrate. Additional studies are needed to investigate insulin response following wild rice consumption and clinical trials using parboiled wild rice to confirm if parboiling process could reduce the impact of wild rice consumption on blood glucose response. By refining the processing methods, such as parboiling, wild rice could become a more accessible and beneficial option for individuals seeking to manage their health through diet.

Bridge to Chapter 5

Chapter 4 showed that the consumption of 140 g of stovetop cooked wild rice increased the postprandial blood glucose response compared to stovetop cooked parboiled white rice in adults and no differences were observed for appetite, despite wild rice having better nutritional profile and similar amount of carbohydrate with cooked parboiled white rice. Numerous studies have demonstrated that parboiled rice helps to regulate the blood glucose concentration than non-parboiled rice. In the clinical trial, the wild rice used was not parboiled but the white rice and brown rice were parboiled. With that, the analyses discussed in chapter 5 were done to compare the nutritional composition of parboiled wild rice and non-parboiled wild rice and its estimated glycemic index in *in vitro* setup.

CHAPTER 5: EFFECTS OF PARBOILING ON WILD RICE NUTRITIONAL COMPOSITION COMPARED TO NON-PARBOILED WILD RICE

5.1 Abstract

Parboiling is a hydrothermal process which involves soaking, steaming and drying paddy before the removal of the rice husk. It results in physical and chemical changes in the rice grains. Hence parboiling reduces starch digestibility, improves its nutritional profile, impact on blood glucose concentration, and shelf life. Several studies have reported that parboiled rice helps regulate the blood glucose response than non-parboiled rice. Our clinical trial reported a high postprandial glycemic response in cooked non-parboiled wild rice compared to cooked parboiled white rice. Therefore, this study's objectives were to investigate and compare the nutritional composition of parboiled wild rice (both cooked and uncooked) and non-parboiled wild rice (both raw and cooked) and determine its estimated glycemic index (eGI) in *in vitro* analysis to help predict glycemic response in humans. Canadian wild rice (*Z. Palustris)* was soaked in water bath at 55 ^{o}C for 1 hour, steamed using autoclave at 100 ^{o}C for 5 min and dried using dehydrator at 35 ^{o}C for 5 hours. It was observed that parboiling improved the protein, total dietary fibre, amylose, total phenolic content, fat content, and resulted in reduction in rapidly digestible starch, slowly digestible starch, total flavonoid content and carbohydrate content in wild rice. Also, the cooked parboiled wild rice showed intermediate eGI of 64 in *in vitro* digestion compared to cooked non-parboiled wild rice (eGI = 77). These outcomes have been reported to be associated with reduced glycemic response. Therefore, this may make cooked parboiled wild rice suitable for the regulation of blood glucose response and enhance production of parboiled wild rice.

5.2 Introduction

Parboiling is a hydrothermal process which involves soaking, steaming and drying paddy before the removal of the rice husk (Balbinoti et al., 2018). Soaking increases the water absorption and moisture content of the paddy especially if warm water is used. It allows the absorption of nutrients (water soluble nutrients) from the rice outer layer (bran) to the endosperm. This can help increase and preserve the rice nutritional component. The soaked rice starch is gelatinized by steaming which also kill microorganisms. Finally, drying is done to reduce the moisture and extend the shelf life of rice. The process of parboiling results in physical and chemical changes in the rice grains that also affect its quality (Kwofie & Ngadi, 2017). Parboiling originated in India and its commonly practiced in many Asian countries and Africa. It was initially employed to reduce breakage of rice grain during milling (Kwofie & Ngadi, 2017). Hence it also reduces starch digestibility, improves its nutritional profile, impact on blood glucose concentration, its quality and shelf life (Balbinoti et al., 2018). To date, parboiled rice (white rice and brown rice) is common in supermarkets across Canada and other countries.

Wild rice is a member of the genus *Zizania.* There are four known *Zizania* species, *Zizania aquatica* L., Zizania *texana* Hitche, *Zizania palustris* L. (found in North America), and *Zizania latifolia* (Griseb) Turcz (found in Asia). They are found naturally in quiet, shallow, and muddy freshwater to brackish waters or lakes that flow gently (Porter, 2019). Wild rice can also be cultivated on set-up beds, which is an artificial set-up that has the environmental conditions necessary for the growth of wild rice (Fyles, 1920; Lu et al., 2005; Surendiran et al., 2014). Wild rice is not usually refined or processed unlike many conventional grains (Surendiran et al., 2014). It is usually harvested, dried naturally under the sun, parched (roast dried), and dehulled (removal of husk) (Fyles, 1920; Surendiran et al., 2014). Raw wild rice has been reported to have higher

protein, dietary fibre, vitamins, phytochemicals, minerals, and lower carbohydrate content, when compared to white rice and brown rice (Saleh et al., 2019; Yu et al., 2020; Zhai et al., 2001). Its consumption has shown numerous health benefits in animals and *in vitro* studies (Deng et al., 2014; Han et al., 2013; Moghadasian et al., 2017; Qiu et al., 2009, 2010; Sumczynski et al., 2017; Surendiran et al., 2013; Wang et al., 2018).

Several studies have reported that parboiled rice can help regulate the blood glucose response compared to non-parboiled rice in both healthy people and people with diabetes (Hamad et al., 2018; Pathiraje et al., 2011). To our knowledge, wild rice has not been parboiled for commercial sales, but research has reported that parboiled wild rice has reduced starch digestibility which is beneficial for blood glucose regulation (Peres et al., 2023). So, from our previous study which investigated the postprandial appetite and blood glucose response to wild rice and wild rice blends in humans, we observed a higher postprandial blood glucose response following the consumption of 140 g of stovetop cooked wild rice. Then it was predicted that since the wild rice used in that study was not parboiled and the white rice and brown rice were parboiled that may have contributed to the difference in outcome. With that, we aim to determine the nutritional composition of parboiled wild rice and its estimated glycemic index (*in vitro*) to help predict the glycemic outcome in humans.

The objective of this study was to investigate the effects of parboiling on wild rice nutritional composition compared to non-parboiled wild rice and estimate potential impacts on blood glucose response. We hypothesized that the parboiled wild rice and cooked parboiled wild rice would have a better nutritional profile than the raw and cooked wild rice respectively but would have lower estimated glycemic index in *in vitro* analysis.

5.3 Materials and Methods

5.3.1 Parboiling process for wild rice

The wild rice was parboiled according to the procedure reported by Peres et al. (2023) with some modifications. Uncooked wild rice (100 g of *Z. palustris* with moisture content of 4.85%) was soaked in 200 mL of distilled water and placed in water bath at 55 ^{o}C for 1 hour. The moisture obtained was 48.47%. Then the wild rice was steamed using autoclave at 100 ^{o}C for 5 min. The sample was stored in fridge at 4.9 ^{o}C for 18 hours. After which the sample was dried using dehydrator at 35 ^{o}C for about 5 hours with stirring at intervals and interchanging the layers of the trays in the dehydrator to achieve uniform drying. After drying, the moisture obtained was 11.42%. The parboiled wild rice was stored in a Ziploc bag at room temperature for 15 days to relief internal stress and stability of the moisture content before performing the other analysis.

5.3.2 Cooking Procedure for Parboiled Wild Rice

In a pot, one cup and a quarter cup of water was added to 65 g of parboiled wild rice. It was brought to boil on medium high heat stovetop for 5 - 6 minutes. Then the heat was reduced to medium low and covered to cook for 30 minutes. After which the heat was turned off and allowed to stand on the hot surface for 10 - 12 minutes.

5.3.3 Analysis of the Parboiled Wild Rice and Cooked Parboiled Wild Rice

The cooked parboiled wild rice was freeze-dried, and both the parboiled wild rice (PWR) and cooked parboiled wild rice (CPWR) were milled through 0.5 mm sieve. The samples were analyzed for moisture, protein, crude fat, total ash, total dietary fibre content, resistant starch, amylose/amylopectin ratio, starch *in vitro* digestion, viscosity, starch damage, total phenolic and flavonoid content as described previously in chapter 4 section 4.3.3.

5.4 Results

Table 8: Nutritional Composition of Raw Wild Rice, Parboiled Wild Rice, Cooked Non-parboiled Wild Rice, and Cooked Parboiled Wild Rice

Nutrients	Raw wild rice	Parboiled wild rice	Cooked non-parboiled wild rice	Cooked parboiled wild rice
Protein (%)	12.8	13.8	13.4	15.5
Carbohydrate (%)	76.1	69.1	76.8	74.0
Moisture (%)	8.6	11.4	4.8	3.9
TDF (%)	4.4	4.7	5.2	6.7
Crude fat (%)	0.9	4.4	3.4	5.2
Ash (%)	1.6	1.3	1.6	1.4
RDS (g/100 g)	23.2	23.3	23.0	16.9
SDS (g/100 g)	53.6	54.1	33.5	31.7
RS (g/100 g)	35.5	29.3	7.7	8.7
Starch damage (%)	NM	12.3	52.7	46.8
TPC (mg GAE /100 g)	46.9	46.2	46.9	54.7
TFC (mg RE/100 g)	267.8	122.0	285.6	123.4
Amylose (%)	31.9	43.0	35.9	44.7
Amylopectin (%)	68.1	57	64.1	55.3
eGI	-	-	77.0	64.0
Viscosity (Pa*s)	NA	NA	1.2	0.9

Key: NA – Not applicable, NM – Not measured, SDS – Slowly digestible starch, RDS – Rapidly digestible starch, RS – Resistant starch, TDF – Total dietary fibre, eGI – Estimated glycemic index**,** TPC – Total phenolic content, GAE – Gallic Acid equivalent, TFC – Total flavonoid content, RE – Rutin equivalent, % db – dry basis, Pa*s – pascals seconds. Results presented in the table are dry basis (db) except for viscosity.

Table 9: Percentage of the nutritional components in 140 g of cooked non-parboiled and cooked parboiled wild rice

Nutrients	Cooked non-parboiled wild rice	Cooked parboiled wild rice
Protein (%)	5.4	5.6
Carbohydrate (%)	33.2	25.5
Fat (%)	1.4	1.9
Moisture	59.4	66.5
Ash (%)	0.6	0.5
TDF (%)	2.1	2.4
SDS (%)	13.4	11.5

RDS (%)	9.2	6.2
RS (%)	3.1	3.2
Energy (Cal/140 g)	67.0	141.5
TPC (%)	0.2	0.2
TFC (%)	1.2	0.5

Key: SDS – Slowly digestible starch, RDS – Rapidly digestible starch, RS – Resistant starch, TDF – Total dietary fibre, TPC – Total phenolic content, TFC – Total flavonoid content

5.5 Discussion and Conclusion

In this study, the nutritional composition of parboiled wild rice and cooked parboiled wild rice was described and compared with uncooked wild rice and cooked non-parboiled wild rice. Generally, there was an increase in the protein, total dietary fibre (TDF), fat, total phenolic content (TPC), and amylose content in the cooked parboiled wild rice sample (Table 8). Most existing literatures have reported the nutritional composition of raw wild rice (Massaretto et al., 2023; Moghadasian et al., 2017; Przybylski et al., 2009; Qiu et al., 2009, 2010; Zhai et al., 2001) and its similar to the measurement seen in the raw wild rice used in this study.

The cooked parboiled wild rice (CPWR) has the highest protein content (15.5%) compared to the other samples. The wastewater from the soaking process was analyzed for protein and 18.7% was observed. This is higher than the protein seen in raw wild rice, cooked, parboiled wild rice and CPWR. Peres et al. (2023) reported that the protein content of parboiled wild rice ranges from 17.78 - 20.92%. In this study, the parboiled wild rice has protein content of 13.8% which is lower than that reported by Pere et al. (2023). This may be due to leaching of some protein into the water used for soaking during the parboiling process.

The lowest carbohydrate content was observed in the parboiled wild rice (69.1%), followed by CPWR (74.0%), raw wild rice (76.1%) and then cooked wild rice (76.8%). Similarly, there was decrease in the rapidly digestible starch (RDS) and slowly digestible starch (SDS) in the cooked

parboiled wild rice (16.9% and 31.7%) respectively compared to raw wild rice, cooked wild rice and parboiled wild rice (Table 8). With this, the starch in CPWR may be digested at a slower rate than that in the cooked non-parboiled wild rice. Peres et al. (2023) also observed reduction in starch digestibility in parboiled wild rice. Hence, there was reduction in the resistant starch (RS) in the CPWR (8.7%) from 29.3% seen in the parboiled wild rice and 35.5% in raw wild rice. Although, the resistant starch in cooked wild rice was also low (7.7%). For total dietary fibre (TDF), the highest 6.7% was in cooked parboiled wild rice followed by 5.2% seen in cooked non-parboiled wild rice. The raw wild rice and parboiled wild rice had 4.4 and 4.7 respectively. Hence, parboiling and cooking increased the TDF in the wild rice.

Qiu et al. (2010) reported the total phenolic compound (TPC) for some raw Canadian wild rice varieties used in their study to be between 438 ± 10 to 585 ± 21 mg GAE/kg (Qiu et al., 2010). In this present study the TPC of raw wild rice is 469 mg GAE/kg (46.9 mg GAE/100 g) which is within the range reported by Qiu et al. (2010). However, the TPC of North American wild rice report by Moghadasian et al. (2017) (762.4 mg GAE/kg) was very high (Moghadasian et al., 2017). The TPC in cooked parboiled wild rice is 54.7 mg GAE/100 g and its higher than that in PWR (46.2 mg GAE/100 g), raw wild rice (46.9 mg GAE/100 g) and cooked wild rice (46.9 mg GAE/100 g) (Table 8). Therefore, cooking did not result in loss or reduction in TPC in the cooked wild rice and cooked parboiled wild rice samples in this study. While for TFC, reduction was observed in the parboiled wild rice (122.0 mg RE/100 g) and cooked parboiled wild rice (123.4 mg RE/100 g) from 267.8 mg RE/100 g in raw wild rice. There was an increase in the TFC in cooked non-parboiled wild rice 285.6 mg RE/100 g (Table 8). Massaretto et al. (2023) reported no effect of cooking on TPC, but reduction in TFC in the wild rice (*Zizania aquatica*) cooked in electric rice cooker in their study (Massaretto et al., 2023). The water used to soak the wild rice

during parboiling was also analyzed for TPC and TFC. It was observed that the wastewater has higher TPC (853.9 mg GAE/100 g), and TFC (2475.6 mg RE/100 g) compared to the parboiled wild rice samples and non-parboiled samples. Subsequently, less water could be used for parboiling to reduce nutrient loss and wild rice with its husk could be used for the parboiling process. This study used dehulled wild rice and this may have contributed to the outcome.

Cooking and parboiling resulted in higher crude fat measurements in the wild rice. The fat content in raw wild rice was 0.9%, cooked wild rice has 3.4%, PWR has 4.4% and CPWR has the highest 5.2%. Peres et al. (2023) reported lower lipid content range from 1.22 - 1.65% in their parboiled wild rice samples. The ash content in parboiled wild rice (PWR – 1.3%) and CPWR (1.4%) was like the ash content range for parboiled wild rice (1.33 - 1.45%) reported by Peres et al. (2023).

Parboiled wild rice and cooked parboiled wild rice have higher amylose content; 43.0% and 44.7% respectively compared to raw wild rice (31.9%) and cooked wild rice (35.9%). Therefore, the process of parboiling wild rice also resulted in an increase in the amylose content. Peres et al. (2023) reported amylose content range between 9.59 - 11.93% for parboiled wild rice with varying pressure and time during the parboiling process (Peres et al., 2023). This is lower than the percentage recorded in this study.

When the percentage of nutritional composition in 140 g of cooked non-parboiled and cooked parboiled wild rice was compared, 140 g of CPWR has lower carbohydrate, total flavonoid content, slowly and rapidly digestible starch than 140 g of cooked non-parboiled wild rice (Table 9). This showed that parboiling can reduce the starch content in wild rice which impacts blood glucose concentration. Both the cooked non-parboiled wild rice and cooked parboiled wild rice showed low viscosity, 1.2 Pa*s and 0.9 Pa*s respectively. With this low viscosity, there will be rapid absorption and digestion of the rice in the intestine which may result in higher blood glucose peak

(Argyrakopoulou et al., 2020; Jin et al., 2023). Also, more starch damage was noticed in cooked non-parboiled wild rice (52.7 %) than in CPWR (46.8 %). Hence, parboiling improves the hardness of the grain which prevents more damage to the wild rice starch (Paraginski et al., 2014; Peres et al., 2023).

From the *in vitro* digestion analysis, the estimated glycemic index (eGI) of the cooked parboiled wild rice was 64 while that of the cooked non-parboiled wild rice was 77. Therefore, the CPWR would be regarded as medium or intermediate eGI food while cooked wild rice is higher eGI food. Based on this analysis, cooked parboiled wild rice may help regulate the blood glucose concentration than cooked wild rice. Therefore, clinical trials should be done to investigate the impact of cooked parboiled wild rice on postprandial glycemic response compared to other parboiled rice (white rice and brown rice).

In conclusion, this parboiling process used for Canadian wild rice (*Z. palustris*) resulted in increase in nutrients such as protein, total dietary fibre, amylose, total phenolic content, crude fat, and reduction in rapidly digestible starch, slowly digestible starch, starch damage, total flavonoid content, and carbohydrate content. These outcomes in the nutritional composition in addition to the intermediate eGI observed in the *in vitro* digestion of cooked parboiled wild rice have been reported to be associated with reduced blood glucose response. Additional studies are needed using wild rice with its husk for the parboiling process and human trials to investigate the *in vivo* postprandial blood glucose response of cooked parboiled wild rice.

CHAPTER 6: CONCLUSIONS

6.1 Research Strengths and Limitations

Some of the strengths of this clinical trial include the fact that it is a randomized controlled trial involving human participants (both male and female participants). The Health Canada's guideline for postprandial glycemic and satiety response were followed, and we evaulated numerous nutritional factors in the study treatments that can impact postprandial appetite and glycemic response. This study did not use rice samples subjected to the same processing conditions such as using all parboiled rice samples or all non-parboiled rice samples for the trial, and insulin response of the participants were not measured. These are some limitations in the study and could have been helpful in explaining the research outcome if measured.

6.2 Research Conclusions

The clinical research showed that acute consumption of 140 g of stovetop cooked wild rice is associated with about 32.7% increase in the blood glucose response compared to stovetop cooked parboiled white rice in adults, despite wild rice having better nutritional profile and similar amount of carbohydrate consumed in both treatments. There were no differences observed for appetite, and white rice and brown rice were more palatable than wild rice and the wild rice blends (15% wild rice and 85% brown rice). Also, there was no difference in the cooking methods (stovetop and microwave) used for wild rice blends on postprandial glycemic response and appetite, despite 140 g of microwave wild rice blend containing 50% more carbohydrate. The nutritional composition of wild rice alone did not account for the differences seen in the study outcome despite cooked wild rice having better nutritional profile than the other rice used in this study. This study also showed that parboiling process can improve the nutritional composition, quality and reduce

the starch content (starch digestibility) of wild rice, therefore this could make parboiled wild rice a better choice in terms of postprandial blood glucose management. This will help inform the development of blended rice products that include wild rice, enhance production of parboiled wild rice and may help inform the selection of rice type to consume for people who want to regulate their blood glucose concentration.

6.3 Future Directions

Clinical trials are needed to investigate the effects of cooked parboiled wild rice on postprandial appetite and blood glucose response compared to cooked parboiled white rice and brown rice. More research is required to determine the long-term consumption of wild rice on appetite, glycemic index and glycemic response in humans. Further studies should be done to match the carbohydrate content in each treatment to ensure similar bases for comparison. Future studies are needed using different population (people with prediabetes and diabetes) since they may show different postprandial glycemic and appetite response.

Furthermore, studies are required to investigate the insulin response following wild rice consumption. Insulin is a hormone that helps regulate the blood glucose concentration by allowing the cells to take up glucose from the bloodstream. The amount of insulin produced can also influence blood glucose concentration (Dimitriadis et al., 2021). Also, hormones such as glucagon-like peptide-1, gastric inhibitory peptide, leptin, ghrelin, peptide tyrosine tyrosine and cholecystokinin can be investigated in wild rice trial as they are involved in appetite and blood glucose regulation.

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CHAPTER 8: APPENDICES

Appendix 1: Research Participant Information and Consent Form (Version 3 – June 1, 2023)

Title of the study: Evaluating the Factors that Influence Glycemic Response to Wild Rice and Wild Rice Blends in Humans (rice blends) study

Principal Investigators:

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Study Sponsors: University of Manitoba

Study Funders: Protein Industries Canada and Mitacs

You are being asked to participate in a research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this study and you may discuss it with your regular doctor, friends and family before you make your decision. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand. Participation in this study is voluntary.

Purpose of Project

The current increase of chronic metabolic diseases such as type 2 diabetes and insulin resistance has led to the search and utilization of foods with low impact on the blood glucose level. Wild rice is a native grain in North America and has been used for food among the indigenous communities for thousands of years. It is a good source of proteins, minerals, vitamins and phenolic compounds that elicit antioxidant activity. Due to its nutritious, and potential health benefits, it shows promise for development into high value food products.

There is no much research on the glycaemic response to wild rice or how the cooking method affects the glycaemic response. Although in 2013, Han et al., studied the effects of dietary carbohydrate replaced with wild rice (*Zizania latifolia* (Griseb) Turcz) on blood glucose level and insulin resistance in rats fed with a high-fat or cholesterol diet. The research concluded that rats

fed with wild rice diet had a lower weight gain and showed a significant reduction in serum fasting insulin and fasting blood glucose concentrations than rats fed with other experimental diets at the end of the experiment. However, after reviewing the previous research done with wild rice on glycaemic response, it was observed that glycaemic response to whole wild rice has not been investigated in humans.

This clinical trial focused on wild rice (North American wild rice), including cooked whole wild rice and blend of 15% wild rice and 85% brown rice compared with whole white rice and whole brown rice. The nutritional content of wild rice has been shown to account for its health promoting benefits so replacing white and brown rice which are commonly consumed by individuals with wild rice would help reduce the occurrence of chronic metabolic diseases. We aim to investigate if the health benefit that was demonstrated in rats (fed with wild rice) can be replicated in humans.

This trial is a part of a larger project titled "An Indigenous Strategy for Re-Energizing Traditional Wild Rice: Indigenous Wealth Creation" funded through Protein Innovation Canada (PIC). This PIC project is led by the Myera Group, which is a Manitoba based Metis company that is developing novel, sustainable biotechnology for producing high-value products that will have a positive impact on human health. The whole wild rice and other rice blends will be provided by the Myera group. This trial consists of five study visits and neither you nor the research team will know which treatment you will be receiving. You will receive one of the five treatments each session in a random order.

Treatments

Served cooked in 140 g servings:

1. Whole white rice (control) Stovetop – ~130 calories
2. Whole brown rice stovetop- ~150 calories
3. Whole wild rice stovetop - ~166 calories
4. 15% Wild rice and 85% brown rice blends stovetop- ~153 calories
5. 15% Wild rice and 85% brown rice blends Microwave- ~153 calories

Study Procedures

You will attend one screening visit. In the screening visit, after we obtain your informed consent form, we will review the inclusion and exclusion criteria with you including measurement of fasting blood glucose (by finger prick) and body mass index (BMI) to determine your eligibility to this study. If you are eligible, you will be booked for your first study session. For this trial, you will complete four study sessions.

During the study sessions you will consume different treatments each session. Study sessions will take place at Richardson Centre for Food Technology and Research (RCFTR) in the University of Manitoba Fort Garry campus and each visit will last about 2.5 hours. Women will be scheduled during the follicular phase of their menstrual cycle (the 2 weeks following menstruation). The break between visits will be a minimum of 3 days.

Before each study session, we will ask you to fast for about 10 - 12 hours, no food or drink will be allowed except for water. Additionally, no alcohol consumption is allowed 24 hours before your session.

At each session, you will be asked to eat a treatment and give finger stick blood samples. The order of the treatments you will receive will be assigned by chance, but you will receive all of them by the end of the study. Finger stick blood samples will be taken to measure blood glucose using a portable glucose monitor. We plan to take 7 finger sticks during each session. The finger stick will be performed by qualified study personnel, who are trained by a registered nurse or physician in proper blood sampling procedure. There is a chance that additional finger stick samples will be taken if there is an issue with the blood glucose reading, however this is rare. Each session will last up to 2.5 hours. You may be asked to return to the RCFTR to repeat a session if any issues arise during the session i.e., feeling dizzy, problems with getting blood glucose readings, etc.

You will be asked to arrive in RCFTR between 7am - 11am for the study sessions. At each session, we will start with measuring your fasting blood glucose by sticking your finger and measuring the glucose value in a drop of blood using a glucometer. If your blood glucose value is over 5.6 mmol/L or less than 3.5, we will have you rest for 10 min before taking another blood glucose measurement. If your blood glucose value is still above 5.6 mmol/L or less than 3.5, you will be rescheduled to come back another day. If your blood glucose is below 5.6 mmol/L and greater or equal to 3.5, we will ask you to consume one of the treatments with a glass of water. You will fill out a palatability questionnaire following consumption of the treatment. After that, we will measure blood glucose at 15, 30, 45, 60, 90 and 120 minutes after your first bite of the product. You will be asked to stay in the clinical area in Richardson Centre for Food Technology and Research (RCFTR) throughout the entire session. You will complete a motivation-to-eat visual analogue scale (VAS) to measure subjective appetite after each blood glucose measure. The questionnaires consist of 100 mm lines with opposing descriptions at either end for each question. Participants mark an “X” on the line to depict their feelings at each time point.

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study staff first.

Risks and Discomforts

As with any study, there may be some risks associated with taking part. You may feel dizzy following the overnight fast, but this is rare. If this happens, you should feel better once you eat the wild rice provided to you. You may have an allergic reaction to the treatment; however, it only contains wild rice, so the risk is low. There is risk and discomfort associated with blood sampling procedure. Great care will be taken when taking your finger stick blood samples. The staff will help you. To make sure that you are not exposed to another person’s needle, we will ask you to sit away from other study participants. We will use disposable finger stick lancets (needles) before taking each blood sample and then put them into the safety container as soon as we are done. There is very little risk of infection from finger stick blood sampling. We will clean your finger with a new alcohol swab before and after each finger stick and will use a new sterile lancet each time. Some discomfort will be felt as a result of a sharp momentary pain caused as the needle enters the skin. However, because the lancet needle is very small the pain felt is usually less than you might feel from skin puncture during vaccination or if a blood sample is taken by a needle inserted in a vein. There might be slight bruising under the skin, but this will be minimized by applying pressure after the finger is stuck and blood glucose is measured.

If you have fasting glucose higher than 5.6mmol/L in two consecutive visits during screening or study visits, we will have to discontinue you from the study. Additionally, if during the study visits, your blood glucose is greater than or equal to 10.0mmol/L or at less than 3.5 mmol/L at 2 hours post product consumption, the PI will review the case with the study physician to discuss the appropriate actions for you.

Benefits

There is no direct medical benefit to you from participating in this study. However, your participation provides valuable information to researchers about the effects of wild rice on blood glucose control.

Costs

There will be no cost to participate in this study.

Reimbursements

You will be compensated for participating in the study, $40 for completing each study session, making a total of $200 by the end of the fifth (5th) study session.

Confidentiality

Information gathered in this research study may be published or presented in public forums, however your name and other identifying information (such as address and email) will not be used or revealed in the publications or presentations. Your personal information and personal health information is being collected under the authority of The University of Manitoba Act. The information you provide will be used for the purpose of this research project. Your personal information and personal health information will not be used or disclosed for other purposes, unless permitted by The Personal Health Information Act (PHIA) or The Freedom of Information and Protection of Privacy Act (FIPPA). If you have any questions about the collection of your personal information or personal health information, contact the Access & Privacy Office (tel. 204-474-9462), 233 Elizabeth Dafoe Library, University of Manitoba, Winnipeg, MB, R3T 2N2.

Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law. The electronic consent form will be signed and stored through an online platform (RedCap) which is managed by the Centre for Healthcare Innovation at the University of Manitoba. PDF copies of your consent will be stored at CDIC or RCFTR either on a password protected shared drive/computer and/or in a locked cabinet. We will employ a unique numerical coding system that will not contain any direct identifiers, such as your name or initials, to de-identify the data collected in this study. All research data collected throughout the study will be inputted into the study database using this unique code. A master list record that will link your name, home and email addresses, phone and participant code will be retained in a locked cabinet in CDIC and/or on a password protected CIDC shared drive. Communication with study personnel may happen through email or phone for the scheduling of study visits, study reminders, and any other s tudy related communications. The study data will not be stored for longer than 7 years following the end of the research project. After this time, all study data will be destroyed. The paper documents will be destroyed using a secure document destruction company and electronic files will be permanently deleted.

Representatives from the University of Manitoba Research Ethic Board may access your confidential records for quality assurance purposes; however, they are committed to confidentiality as well. In addition to the U of M Research Ethics Board, authorized representatives of Agriculture and Agri -Food Canada may inspect and/or copy de-identified research records for quality assurance and data analysis.

The data collected from you during this study may be shared in a de-identified form to academic journals for publication purposes, or academic researchers for the purpose of meta-analysis and further data analysis. The data may also be stored by the academic journal or other open-access repositories under an open access policy in which case it may be used by other researchers for further data analysis and research purposes.

All physical records will be kept in a locked secure area in the Chronic Disease Innovation Center (CDIC), Seven Oaks Hospital or RCFTR) and only those persons on the research team or identified will have access to these records. The paper file that links your personal and contact information to numerical coding system will be stored in a separate locked cabinet in CDIC. Your name and address will be used during the trial to deliver the trial interventions, remuneration cheques and material to your house, both by study staff and/or contracted delivery personnel. Electronic records of signed consent forms as well as your email address will be stored on REDCap, as well as stored in a password protected secure computer/shared drive at CDIC. No personal identifying information such as your name, address, or contact information will leave Chronic Disease Innovation Center, except as described above.

This clinical trial is registered on a publicly available registry databank: Clinicaltrials.gov. This is a website that provides information about federally and privately supported clinical trials. A description of this clinical trial will be available on http://clinicaltrials.gov. This website will not include information that can identify you. At most, the website will include a summary of the results which have been de-identified. You can review this website at any time.

Voluntary Participation/ Withdrawal from the Study

Your decision to take part in this study is voluntary. You may refuse to participate, or you may withdraw from the study at any time. If the Principal Investigator feels that it is in your best interest to withdraw you from the study, the Principal Investigator can remove you without your consent.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

Questions

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study or if you have a research-related injury, contact the study staff:

Principal Investigator: Dr. Dylan MacKay	dylan.mackay@umanitoba.ca
Principal Investigator: Rebecca Mollard	rmollard@sogh.mb.ca
Study physician: Dr. Navdeep Tangri	ntangri@sogh.mb.ca

Research Coordinator: Dianna Dandeneau ddandeneau@sogh.mb.ca

For questions about your rights as a research participant, you may contact The University of Manitoba Biomedical Research Ethics Board at (204) 789-3389.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all your questions.

Statement of Consent

1. I have read this consent form. I have had the opportunity to discuss this research study with Dylan MacKay and/or his study staff.

1. I have had my questions answered by them in language I understand.

2. The risks and benefits have been explained to me.

3. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statement or implied statements.

4. I have no relationship (such as employee, student or family member) with the study team.

5. I understand that I will be given a copy of this consent form after signing it.

6. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time.

7. I freely agree to participate in this research study.

8. I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed.

9. I hereby consent to undergo all necessary assessments that may be required in the course of this study.

10. I authorize the inspection of my research records by the University of Manitoba Research Ethics Board.

11. I consent to my address being used and provided to a delivery/courier service for the delivery of the study related materials and remuneration cheques.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature Date (day/month/year)

Participant printed name:

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: Date (day/month/year)

Signature:

Role in the study:

Appendix 2: Screening Assessment

Screening ID:

Date of screening:

Age:

Sex at birth:

Height:

Weight:

BMI (Kg/m^2)

Fasting blood glucose:

Appendix 3: Eligibility Criteria

Date form completed:

Completed by (Staff name):

Section header: Inclusion criteria to meet inclusion criteria, ALL items must be "YES"

Aged 18 – 50 years yes/no

Able to give written informed consent and able to speak/read English? yes/no

BMI range between 18.9 - 29.9kg/m^2 yes/no

Fasting blood glucose ≤ 5.6 mmol/L yes/no

Participant usually eats breakfast? yes/no

Section Header: Exclusion criteria to meet exclusion criteria, ALL items must be "NO"

1. Participant has an existing relationship with research team such as supervisory relationship (student, employee) or familiar relationship (child, spouse etc)? yes/no

2. Participants who indicate that they could not finish study treatment within 10 minutes? yes/no

3. Fasting blood glucose ≥ 5.6 mmol/L? yes/no

4. Female participant who is pregnant, lactating or planning pregnancy during the course of the trial? yes/no

5. History of AIDs, hepatitis, a history of clinically important endocrine (including Type I and II diabetes mellitus) cardiovascular (including but not limited to atherosclerotic disease, history of myocardial infarction, peripheral arteria disease, stroke), pulmonary, biliary or Gi disorders? yes/no

6. Use of medication to influence carbohydrate metabolism, including not limited to adrenergic blockers, diuretics, thiazolidinediones, metformin and systemic corticosteroids within 4 weeks of screening visit? yes/no

7. Intolerance or allergic reaction to study treatments? yes/no

8. Extreme dietary habits (Atkins diet, very high protein diet etc)? yes/no

9. History of hypertension yes/no

10. History of cancer for the last two years (except for non-melanoma skin cancer)? yes/no

11. Participating in another interventional trial that can influence the intervention or outcome of this trial? yes/no

12. Recent history (within 12 months of screening) or strong potential for alcohol or substance abuse. Alcohol abuse is defined as > 14 drinks per week, (1 drink = 12oz of beer. 5oz of wine or 1.5oz distilled spirits)? yes/no

13. Body weight change over 3.5kg in the past 3 months? yes/no

14. Have diabetes or thyroid problems or other major diseases that may affect glucose response? yes/no

15. Have major trauma or surgical event within 3 months of screening? yes/no

16. Participant is eligible for the trial? yes/no

17. Please comment on why participant is not eligible:

Date screening form completed

Completed by (staff name):

PI signature:

Appendix 4: Presession checklist

Date of visit:

1.	Is participant repeating a treatment in this visit?	yes/no
2.	Select which treatment is being repeated	A,B,C,D,E
3.	Fasted over the last 12 hours?	yes/no

Participant is not fasted. Please DO NOT proceed with this visit

4.	Had alcohol in the last 24 hours?	yes/no
5.	Had a normal/typical night sleep?	yes/no
6.	Any unusual stress?	yes/no
7.	Participated in any vigorous activity this morning?	yes/no
8.	Any changes to medications since last visit/last recorded?	yes/no
9.	Can participant proceed with this visit?	yes/no

Appendix 5: VAS Appetite

Time survey started: 0mm (very weak) to 100mm (very strong)

1.	How strong is your desire to eat?	0 mm to 100 mm
2.	How hungry do you feel?	0 mm to 100 mm
3.	How full do you feel?	0 mm to 100 mm
4.	How much food do you think you could eat?	0 mm to 100 mm
5.	How thirty do you feel?	0 mm to 100 mm

Complete?

Appendix 6: VAS Palatability

1.	How pleasant have you found the beverage/food?	0 mm to 100 mm
2.	How tasty have you found the treatment?	0 mm to 100 mm
3.	How did you like the texture of the treatment?	0 mm to 100 mm

Complete?